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A Dissertation
for the Degree of Doctor of Philosophy

Effects of Imprinting Impacts through Supplementation of Star Anise during Pre- or Postnatal Period on Performance of Sows and Their Progeny

분만 전후 스타 아니스 급여를 통한 각인효과가 모돈 및 자돈 성적에 미치는 영향

August, 2017

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Effects of Imprinting Impacts through Supplementation of Star Anise during Pre- or Postnatal Period on Performance of Sows and Their Progeny

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이 논문을 농학박사 학위논문으로 제출함

2017 년 8 월

서울대학교 대학원 농생명공학부

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Overall Summary

Effects of Imprinting Impacts through Supplementation of Star Anise during Pre- or Postnatal Period on Performance of Sows and Their Progeny

The objectives of these experiments were 1) to investigate the effects of dietary star anise (*Illicium verum,* SA) supplementation during gestation and lactation on the performance of multiparous sows and their progeny until 21 days post-weaning, 2) to evaluate the effects of dietary SA supplementation during late gestation and post-weaning on the performance of multiparous sows and growth performance of their progeny, and 3) to investigate the effects of SA supplementation in growing to finishing pigs after prenatal exposure of SA during late gestation on growth performance and meat quality in pigs.

Experiment I. Effects of Dietary Star Anise (*Illicium verum*) Supplementation during Late Gestation and Lactation on the Performance of Multiparous Sows and Their Progeny until 21 Days Post-weaning

This study was conducted to investigate the effects of star anise (SA) supplementation during late gestation and lactation on the performance of multiparous sows and their progeny until 21 d post-weaning. A total of 40 pregnant sows [Yorkshire × Landrace] were housed in an individual stall and allocated on the basis of body weight (BW), backfat thickness (BFT) and parity in a completely randomized design (CRD) with one of two treatments, either 0% or 0.1% SA supplementation. After lactation period, a total of 160 weaning pigs were used to

investigate imprinting effect of SA on growth performance and antioxidant status. Dietary treatments were equally allocated as their litter. In late gestation, sows fed SA showed significantly higher serum total antioxidant status (TAS) activity (P=0.03) and a tendency of increased lactation average daily feed intake (P=0.08). When sows were fed SA during late gestation tended to increase litter weight at 21 d of lactation as well as significantly increase litter weight gain (P=0.02, P=0.04, respectively). Sows consumed SA in late gestation tended to have lower protein in sow milk (P=0.07). In contrast, supplying SA in gestation diet resulted in increasing lactose content in the milk (P<0.01). Gestation × lactation effect was noted in the total solid and free fatty acid (FFA) concentration of sow milk at 21d lactation (P=0.06, P=0.04, respectively). At weaning, supplying SA diet during late gestation reduced concentration of serum cortisol in piglet and epinephrine at weaning (P=0.01, P=0.04, respectively). Moreover, there was a gestation × lactation interaction in serum cortisol concentration of piglet (P=0.01). Supplementing SA during late gestation tended to enhance gain to feed (G:F) ratio of weaning pigs at one and two weeks (P=0.08). Similarly, supplementing SA during lactation increased gain to feed ratio both in two and three weeks and overall period (P=0.03, P=0.05, respectively). The SA supplementation during lactation reduced serum cortisol concentration of weaning pigs in initial (P=0.05). Main effects of gestation, lactation, and gestation × lactation interactions were noted in serum superoxide dismutase (SOD) activity at initial and three week of weaning pigs (P<0.01, respectively). Therefore, our results suggested that inclusion of SA in gestation and lactation diet showed higher serum antioxidant properties in sows and increased lactose and free fatty acid in sow milk, consequently enhancing litter weight and feed efficiency at 21 d post-weaning.

Experiment II. Effects of Star Anise (*Illicium verum*) Supplementation during Late Gestation and Post-weaning on Performance of Sow and Their Progeny

The present study was conducted to investigate the effects of star anise (SA) supplementation during late gestation and post-weaning on the performance of multiparous sows and their progeny. A total of 50 pregnant sows [Yorkshire × Landrace] were housed in an individual stall and allocated on the basis of body weight (BW), backfat thickness (BFT) and parity in a completely randomized design (CRD). Sows were offered SA treatment diets with 0.1% SA or control diet during late gestation. After lactation period, a total of 120 weaning pigs were used to investigate imprinting effect on weaning pigs' growth performance. Piglets were fed treatments diet with 0.02 or 0.04% SA after weaning. In late gestation, there were no significant differences in physiological responses in relation to the effect of supplementing SA. There were no significant differences in number of piglets, litter weight change, and piglet weight during lactation period. When sows were fed control diet, high level fat contents and total solid in sow milk at 21d lactation were observed (P=0.01, respectively). Supplementing 0.04% SA during post-weaning tended to enhance ADG at two to four weeks (Weaning, P=0.09). Additionally, prenatal exposure during late gestation group showed lower ADFI at initial time to one week (Gestation, P=0.09). Supplementing 0.02% SA during post-weaning increased gain to feed ratio significantly at 0-1 week (Weaning, P=0.02). Prenatal exposure of SA improved the CV of piglets at weaning (Gestation, P=0.02) and 4 week after weaning (Gestation, P<0.01). Serum cortisol levels tended to be lower at 4 weeks after weaning for prenatal exposure piglets to control piglets (GxW, P<0.01). These results suggested that inclusion of SA in gestation and re-exposure through post-weaning diets enhanced uniformity of piglets as well as reduced stress level of piglets. And less than 0.02% SA supplementation in post-weaning diets recommended to observe imprinting impacts in weaning pigs.

Experiment III. Effects of Star Anise (*Illicium verum*) Supplementation during Growing to Finishing Periods after Prenatal Exposure of Star Anise on Growth Performance and Meat Quality in Pigs

The effects of prenatal and postnatal exposure with star anise (SA) flavor for young animals have been reported. However, the information on long term effects of re-exposure of flavor after prenatal exposure and meat quality impacts by long term supplementation of SA flavor is lacking. To investigate long term effect of SA in meat quality, a total of 120 growing pigs ([Yorkshire × Landrace] × Duroc), averaging 24.83±2.95 kg body weight were used in feeding trial. The experimental design was composed by two factors with factorial design for evaluating imprinting effects on growing-finishing pigs. The first factor was SA supplementation (0% or 0.1%) in late-gestation period of sows and the second factor was SA supplementation (0% or 0.02%) in growing-finishing period. There were no significant difference in body weight (BW) and average daily feed intake (ADFI) in growing to finishing phase (0-13week). However, ADG was increased as prenatal exposure and supplementation level of SA during growing-finishing period in 11-13week (GxL, P=0.03) and 8-13 week (GxL, P=0.04). In addition, there was a increase gain to feed ratio (G:F ratio) as prenatal exposure in 8-13week (Gestation, P=0.03) and overall period (Gestation, P=0.02). Prenatal exposure and re-exposure of SA flavor group showed improvement 46.67% of uniformity than control group (Gestation, P=0.05). Plasma cortisol was higher at prenatal SA flavor than control diets (Gestation, P=0.02) at 3 week. However, control diet treatment showed higher cortisol level at 12 week (Gestation, P=0.02). Prenatal SA flavor treatment showed higher superoxide dismutase (SOD) than control diet treatment at 9 week (Gestation, P<0.01). The lower pH of pork on 24 hour postmortem of carcass as prenatal exposure of SA through maternal diets observed (Gestation, P=0.03). Crude protein of pork decreased (Gestation, P=0.03) as prenatal impacts and crude fat increased as prenatal impacts (Gestation, P=0.03). The result suggested that supplementation SA during late gestation and re-exposure of SA flavor or supplementation during growing-finishing pigs improved growth performance of pigs without negative impacts of pork quality.

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List of Abbreviation

AA	:	Amino acid
ADG	:	Average daily gain
ADFI	:	Average daily feed intake
AF	:	Amniotic fluid
AHDB	:	Agriculture and Horticulture Development Board
AI	:	Artificial insemination
AOAC	:	Association of official analytical chemists
ATTD	:	Apparent total tract digestibility
BFT	:	Backfat thickness
BW	:	Body weight
CP	:	Crude protein
CPK	:	Creatine phosphokinase
CRD	:	Completely randomized design
CV	:	Coefficient of variation
DM	:	Dry matter
FDA	:	Food and Drug Administration
FFA	:	Free fatty acid
G/F	:	Gain to feed
G:F ratio	:	Gain to feed ratio
GPx	:	Glutathione peroxidase
NRC	:	National Research Council
ME	:	Metabolizable energy
MSY	:	Market per sow per year
PSY	:	Pig per sow per year
RCB	:	Randomized complete block
ROS	:	Reactive oxygen species
SA	:	Star anise

SAS	:	Statistical Analysis System
SBM	:	Soybean meal
SD	:	Standard deviation
SFA	:	Saturated fatty acid
SOD	:	Superoxide dismutase
TAS	:	Total antioxidant status
UFA	:	Unsaturated fatty acid
USDA	:	United States Department of Agriculture
WEI	:	Wean to estrus interval
WHC	:	Water holding capacity

Chapter I.General Introduction

Korean pork industry grew up the second largest gross product in agriculture and livestock industry in Korea (MAFRA, 2015). The gross product of pork was 3.6 trillion won in 2006, and it grew up 6.6 trillion won in 2014 (Figure 1). It was the second largest amount of production in agriculture industry, after rice production in 2014.

Despite such quantitative growth of industry, pig production industry faced several challenges yet. Price competitiveness of Korean pork became more difficult because Korean government expanded the FTA with major economies such as the US, Europe and China (Kim et al., 2017). Additionally, the outbreak of foot-and-mouth disease, wasting disease and environmental regulation also affect the price competitiveness of Korean pork (Kim et al., 2017). For these reasons, improvement of pork productivity is very important issue in Korean pork industry to grow sustainably.

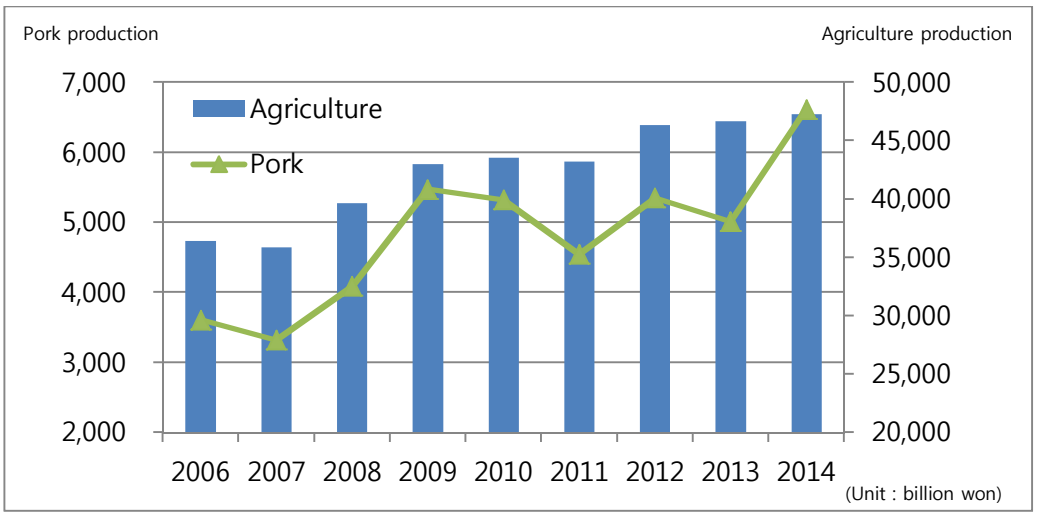

Figure 1. Historical gross production of pork in Korea (adapted MAFRA, 2015)

Compared with the productivity of pigs in major countries, the productivity of pigs is still low in Korea (Table 1). According to the survey of Agriculture and Horticulture Development Board (AHDB), average of market per sow per year (MSY) in European countries was 25.13 in 2014. On the other hand, MSY in Korea was 17.9 in 2014. In case of pig per sow per year (PSY) of other countries reached more than 24, however Korea still remained 20.8. These indicators showed that there are still many areas where the Korean pig industry needs to make efforts to improve productivity.

Table 1. The productivity of pigs in major countries (adapted AHDB, 2016)

Country	Korea	EU	US	Denmark	Netherlands	France	Spain	Brazil
PSY	20.8	26.53	24.6	30.5	29.2	27.4	25.8	26.09
MSY	17.9	25.13	22.4	28.5	27.8	25.7	24.2	25.01
Finishing mortality (%)	-	2.59	4.85	3.70	2.30	3.59	3.45	2.20

As one of the ways to increase pig productivity, many European countries considered phytogenic feed additives after ban of antibiotic growth promoters in 2006 (Steiner, 2009). Phytogenic feed additives are compound derived from leaves, flowers, roots or other parts of plants used in animal feeding for improving the performance of economic animals (Windisch et al., 2008). The effect on microbial inhibition of phytogenic substances is very well known in vitro studies (Smith-Palmer et al., 1998; Si et al., 2006; Windisch et al., 2008). This efficacy has also found in vivo studies with piglets (Manzanilla et al., 2004; Namkung et al., 2004). Also previous researches showed that phytogenic feed additives may help to; improve feed intake from the flavor and odor (Jacela et al., 2010); antioxidative effects from certain compounds (Padmashree et al., 2007). Recently pork producers and researchers have sought new areas from these phytogenic feed additives for improving pork productivity and animal welfare (Steiner, 2009).

Studies in several species, such as humans and dogs have shown that prenatal or postnatal exposure to specific flavors from the maternal diets may affect

to a preference for these flavors when they take food/feed during neonatal period (Schaal et al., 2000; Hepper and Wells, 2006). Some studies have been actively conducted to reduce the stress and increase the feed intake in the weaning pig by utilizing such a mechanism. Piglets weaned at 3 to 4 weeks of age are insufficiently accustomed to the intake of solid feed (Langendijk et al., 2007). Therefore, weaning piglets are underfeeding 1~ 2 days and insufficient nutrient intake during the 1st to 2nd days after weaning is a major factor in the early growth stagnation (Bruininx et al., 2002; Langendijk et al., 2007).

Anise oil and trans-anethole are very common plants derived flavor used in food industry (Wang et al., 2011). And it was used for prenatal and postnatal studies of Langendijk, et al., (2007) and Oostindjer et al., (2009, 2010, and 2011). Prenatal or postnatal exposure of anise flavor in sow and pig may have positive effects on feed intake, growth performance and behavior of piglets during post-weaning period (Langendijk, et al., 2007; Oostindjer et al., 2009, 2010, 2011). However, the effects of anise flavor on growth performance of piglets were not consistent by each study.

Consequently, to increase growth performance of pigs by reducing stress through prenatal or postnatal learning and re-exposure of star anise (SA) flavor and supplementation, three experiments were conducted to investigate 1) the effects of dietary SA supplementation during gestation and lactation on the performance of multiparous sows and their progeny until 21 days post-weaning, 2) the effects of dietary SA supplementation during gestation on the performance of multiparous sows and supplementation levels during post-weaning on the performance of their progeny, and 3) the effects of SA supplementation during growing to finishing periods after prenatal exposure of SA flavor during late gestation on growth performance and pork quality of pigs.

Chapter II. Review of Literature

1. Growth performance and voluntary feed intake

1.1 Feed intakes in post-weaning pigs

The transition from suckling milk (sow's milk is 80% water and the dry matter (20%) is composed of protein (30%), fat (40%), and lactose (25%)) to the solid feed (usually post weaning diet is 90% dry matter and is composed of starch with protein, fat, lactose, etc.) is a challenge for piglets after weaning in commercial pig farm, which are usually weaned at a young age (Williams, 2003; Bolhuis et al., 2009). With this changing of feed physical form and several stressors (separate from sows, transition to new environment and social stress from mixing with unfamiliar piglets) from environmental change interrupt timely and sufficient intake of nutrient during the immediate post-weaning period (Bolhuis et al., 2009).

According to the research of Le Devidich and Sève (2000), feed intake of piglets, deprived of sow milk provided by their lactation sow and offered a solid feed after weaning is often very low. This feed intake reduction affects the total energy intake of piglet as a result. Figure 1 shows that the level of metabolizable energy (ME) intake attained at the end of the 1st post weaning week ranged from 700 and 800 kJ ME/$kg^{0.75}$ (Le Devidich and Sève, 2000). It is just 60~70% of ME intake through sow milk during pre-weaning period.

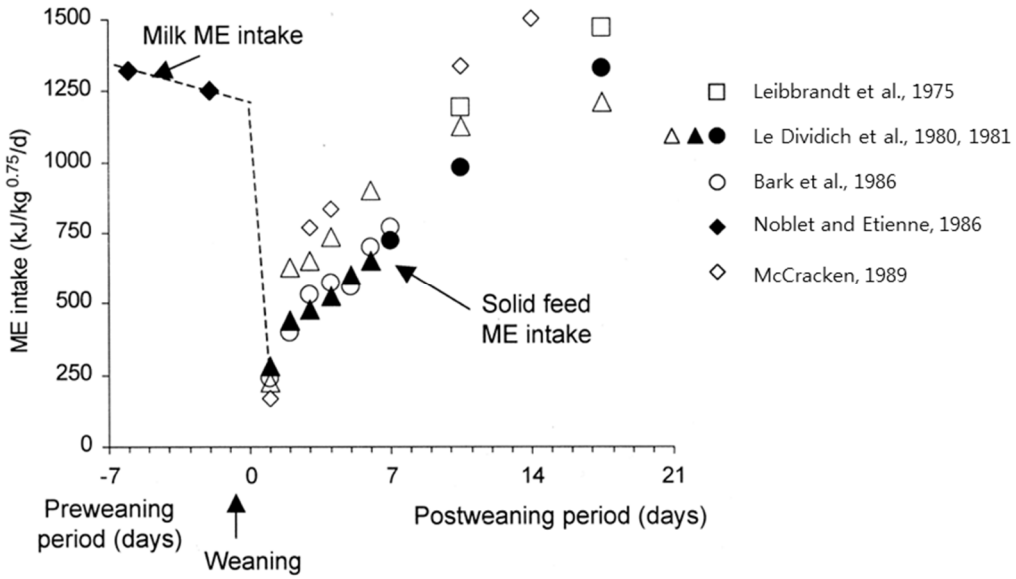

Figure 1. Effect of weaning between 3 and 4 weeks of age on voluntary metabolizable energy intake in piglets (Le Dividich and Sève, 2000).

In the study of Carroll et al. (1998), serum cortisol was evaluated to determine whether weaning or the change in post-weaning diet would be associated with stress or metabolic responses during post-weaning period. In this report, cortisol values increased significantly in newborn pigs due to fasting, and dietary change (milk to phase 1 diet and phase 1 to phase 2 diets) was associated with consistent changes in cortisol concentrations. In this study showed that dietary change (phase 1 to phase2) influenced feed intake and growth performance of piglets not only changing from milk to solid feed.

Tokach et al. (1992) reported importance of weight gain during the first week post-weaning. In this study, piglets gaining more than 226g (0.5lb)/d during the first week post-weaning obtained 7.7kg (17lb) more weight gain at 156d after weaning. This result show that feed intake and weight gain during post-weaning period is important because they influence on subsequent growth of pigs (Tokach et al., 1992; Collins et al., 2017).

1.2 Feed intake in growing-finishing pigs

Growing-finishing pigs are provided feed *ad libitum* basically in a typical commercial farm. Therefore, total amount of feed consumed during growing-finishing period is very important factors to determine their growth performance, live weight and tissue accretion rates (Ellis and Augspurger, 2001). Several environmental factors (temperature, humidity, air circulation nutrition, health, other pigs, internal factors, dietary factors, etc.) are changed frequently during growing-finishing period (Rose and Kyriazakis, 1991; Nyachoti et al., 2004; Knap, 2009). These environmental factors can be potential constraints of feed intake during growing-fishing period (Nyachoti et al., 2004).

While numerous researches have generally studied the impact of the environment on growth performance in the way of isolated individual factors (Knap, 2009), Hyun et al. (1998) reported that there are multiple stressors (high ambient temperatures, reduced floor space and regrouping) singly and multiply impact on growth performance of growing pigs. In this report, multiple stress factors depressed 5-7% average daily feed intake during experiment period. Generally domesticated pigs are able to ingest feed sufficiently for meeting their apparent requirement in proper conditions (Rose and Kyriazakis, 1991). However, pigs are exposed to multiple stressors in general commercial farms. Therefore, reducing stress factors and relieving stress are very important for improving growth performance of growing-finishing pigs.

1.3 Prenatal learning process and feed preference

Prenatal learning is that mammals develop a specific food preference by exposure of specific flavors during prenatal period from maternal diet (Charal, 2015). Studies in several species, such as humans and dogs have shown that prenatal exposure to specific flavors from the maternal diets may affect to an olfactory preference for these flavors when they take foodstuff during neonatal period (Schaal et al., 2000; Hepper and Wells, 2006). To prove the hypothesis of prenatal olfactory learning, a variety of studies approaches have been developed

over the past four decades to manipulate the chemosensory environment of the fetus and to evaluate the effects on growth performance and behavioral features after birth (Robinson and Méndez-Gallardo, 2010).

Prenatal olfactory learning has been studied in rats with apple juice: [Smotherman, 1982], citral; [Perdersen & Blass, 1982], garlic; [Hepper, 1988], ethanol; [Chotro & Molina, 1990], ethanol; [Dominguez et al,. 1998] and ethanol; [Arias & Chotro, 2005]), rabbits (juniper berries; [Bilkó et al., 1994], juniper berries; [Semke et al.,1995] and black cumin; [Coureaud et al,. 2002]), dogs (aniseed; Wells and Hepper, 2006), humans (anise; [Schaal et al., 2000] and carrot [Mennella et al., 2001]), lamb (oregano essential oil; [Simitzis et al., 2008] and citral; [Schaal et al., 2008]), and pigs (garlic and anise; Langendijk et al., 2007), where flavors in the maternal diet lead to a postnatal preference for these flavors (Blavi et al., 2016). Most of the researches have focused on the short-term periods after birth and studied preference behavior for food. The few studies (Langendijk et al., 2007; Oostindjer et al., 2010, 2011; Blavi et al., 2016) that have investigated the effect of prenatal exposure on growth performance in pigs. Table 1 show that summary of prenatal learning studies with method of prenatal exposure, method of postnatal test and test results from progeny after birth (Robinson and Méndez-Gallardo, 2010).

The most important factor in the prenatal learning process is the amniotic fluid (AF) (Mennella et al., 2001; Browne, 2008; Blavi et al., 2016). According to the Robinson and Méndez-Gallardo (2010), during the late gestation, the composition of AF is the combined product of maternal diet and physiology, and the physiology and behavior of the fetuses. Fetal swallowing and micturition influence the composition exchange between fetuses and AF during the late gestation period (Mennella et al., 2001; Browne, 2008; Robinson and Méndez-Gallardo, 2010; Blavi et al., 2016).

According to the study of Blavi et al. (2016), anethole, cinnamaldehyde, and eugenol were detected in amniotic fluid from sows fed treatment diet with complex flavored (anethole, cinnamaldehyde, eugenol) from 73 days to farrowing. In this study, authors explained that prenatal exposure through amniotic fluid may

be a powerful pathway for learning of maternal flavor to newborn piglets and be enough to establish a linkage between prenatal experiences and the post-weaning period. Also, prenatal exposure of certain flavors from maternal diets enhances feed intake and growth performance of offsprings (Oostindjer et al., 2009, 2010, 2011; Blavi et al., 2016).

As a result of these previous studies, prenatal exposure of phytogenic feed additives which extracted from plant materials can help to enhance voluntary feed intake after weaning and growing-finishing period through re-exposure or supplementation of same flavor materials in maternal diets.

Table 1. Studies of fetal exposure learning, based on prenatal chemosensory exposure and postnatal behavioral assessment (Robinson and Méndez-Gallardo, 2010).

Species	Prenatal exposure			Postnatal testing				Citation
	Stimulus presented	Method of exposure	Age of exposure	Stimulus presented	Age of testing	Method of assessment	Results	
Rat (*Rattus norvegicus*)	Apple juice	Direct injection into AF	Day 20	Apple juice vs. water	Day 60 after birth	Two-bottle preference drinking test	Greater intake of apple juice	Smotherman, 1982
	Citral	Direct injection into AF	Day 20	Citral vs. nothing	Day 1 after birth	First nipple attachment	Pups attached to nipple coated with citral	Pedersen, & Blass, 1982
	Garlic	Females fed garlic	Day 15 to Day 21 of gestation	Garlic vs. onion	Day 12 after birth	Double-choice paradigm : Pups moved toward a side	Preference for garlic	Hepper, 1988
	Ethanol	Direct injection into AF	Day 21 of gestation	Ethanol and lemon	Day 8 after birth	Odor preference test and intake test	Preference for ethanol odor and greater intake	Chotro, & Molina, 1990
	Ethanol	Maternal intragastric intubation	Day 17 to Day 20 of gestation	Ethanol, water, sucrose, quinine, and sucrose mixed with quinine	Day 14 after birth	Intake test	Greater intake of ethanol and sucrose mixed with quinine	Domínguez, López, & Molina, 1998

Species	Prenatal exposure			Postnatal testing				Citation
	Stimulus presented	Method of exposure	Age of exposure	Stimulus presented	Age of testing	Method of assessment	Results	
Rabbit (*Oryctolagus cuniculus*)	Ethanol	Maternal Intragastric intubation	Day 17 to Day 20 of gestation	Ethanol, sucrose + quinine, water	Day 14 after birth	Taste reactivity test and intake test	More ingestive responses and greater intake of ethanol	Arias, & Chotro, 2005
	Juniper berries	Females fed juniper berries	From day 15 of pregnancy until day 28 after birth	Juniper berries vs. lab chow or water	Day 28 after birth	Feeding preference test	Preference for juniper berries after prenatal and postnatal exposure	Bilkó, Altbäcker, & Hudon, 1994
	Juniper berries	Females fed juniper berries	From mid gestation	Juniper berry odor vs. nothing	Day 1 after birth before first suckling	Double-choice olfactory test in an arena	Preference for the odor of juniper berries	Semke, Distel, & Hudson, 1995
	Black cumin	Females fed food and water mixed with cumin	From day 17 of pregnancy until day 4 after birth	Placenta, colostrum, garlic, and cumin	Between day 1 and day 3 after birth	Two-choice olfactory test and oral activation test (searching and grasping)	Preference for cumin in all tests	Coureaud, Schaal, Hudson, Orgeur, & Coudert, 2002
Domestic dog (*Canis lupus familiaris*)	Aniseed	Females fed diet containing aniseed	Last 20 days of gestation	Aniseed vs. water, vanilla vs. water	Day 1 after birth	Head turning preference test	Pups oriented their head toward the odor of aniseed	Hepper & Wells, 2006

Species	Prenatal exposure			Postnatal testing				Citation
	Stimulus presented	Method of exposure	Age of exposure	Stimulus presented	Age of testing	Method of assessment	Results	
Human	Anise	Mothers fed anise flavored food	Week 39 and 40 of gestation	Anethole (pure anise flavor) diluted in paraffin oil vs. paraffin oil	Day 1 and Day 4 after birth	Oral and facial responses and Head orientation	Less negative facial expressions and head orientation toward anise	Schaal, Marlier, & Soussignan, 2000
	Carrot juice	Mothers drank carrot juice	Last trimester of pregnancy and first 2 months of lactation	Cereal with carrot juice vs. cereal with water	5 mo after birth	Negative facial responses and mother's reports	Less negative facial expression for carrot cereal	Mennella, Jagnow, & Beauchamp, 2001
Pig (*Sus domestica*)	Garlic and Anise	Females fed diet containing garlic or anise	Last month of gestation and during lactation	Garlic and Anise in food vs. normal food	Days 3 and 10 after weaning	Feed intake	Greater feed intake by piglets weaned at 6 weeks and after prenatal exposure	Langendijk, Bolhuis, & Laurenssen, 2007
Lamb (*Ovisaries*)	Oregano essential oil	Females fed diet containing oregano essential oil	Between days 50 and 130 of pregnancy	Food with oregano, orange or eucalyptus essential oils	Day 45 after birth	Feeding preference test	Preference for food with oregano essential oil	Simitzis, Deligeorgis, Bizelis, & Fegeros, 2008
	Citral	Females fed diet containing citral	Last 2 weeks of pregnancy	Citral vs. AF	Day 1 after birth	Head turning preference test	No preference	Schaal, Orgeur, & Arnould, 1995

1.4 Postnatal learning process and feed preference

Young animals acquire information about feed items through a trial and error learning process or social information transmission from mother to ingest feed alone (Bolhuis et al., 2009). These learning processes of their diets may help to avoid neophobia during diet change from milk to solid feed. According to the report of Beauchamp and Mennella (2009), breastfeeding such as human milk provides oral sensory experiences to young animals like as prenatal experiences through amniotic fluid. Mennella et al. (2001) reported that infants who had exposure to the carrots flavor through mother's milk response differently to solid food which have same flavor than non-exposed control infants. In the study of Hausner et al. (2008), milk composition of d-carvone and trans-anethole increased sharply 2 hours after ingesting flavor capsule by mothers (Figure 2). On the other hand, l-menthol did not change through ingestion and 3-methilybutyl acetated did not detected from mother's milk. These studies suggest that milk is strong pathway of certain flavor transmission from dam to their progeny during lactation periods. However, in order to be transformed to milk from maternal diet, the type of flavor and the amount of intake seem to be important (Hausner et al., 2008; Blavi et al., 2016).

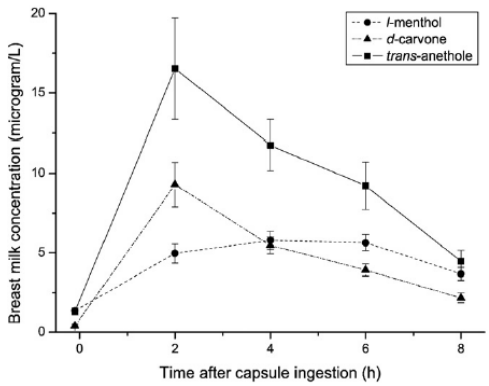

Figure 2. Average concentrations of l-menthol, d-carvone and trans-anethole in breast milk from lactating women after ingestion of 100mg doses at time 0 h on three separates test days. Values are means of the 3 days±SEM, n=18. (Hausner et al., 2008)

Postnatal olfactory learning has been studied in rats (lab chow; [Galef and Sherry, 1973], lab chow; [Galef and Henderson, 1972], lab chow; [Bronstein et al.,1975], garlic; [Capretta and Rawls, 1974] and onion; [Wensch, 1978]), mice (fennel ; [Mainardi et al., 1989]), rabbits (junifer; [Bilkó et al., 1994]), dogs (anise; [Hepper and Wells, 2006]) and humans (carrot; [Menella et al., 2001 and Menella and Beauchamp, 1999]). Most of studies used 2 or 3 way food choice test to evaluate effects of postnatal exposure through maternal diet on feed preferences of their progeny (Bolhuis et al., 2009). However, there is no selective feeding in a typical farm environment, it is important to study impacts of postnatal flavor exposure on growth performance with single diet. Also, Hepper and Wells (2006) reported that postnatal exposure of anise flavor is less effective than perinatal exposure in feed preferences. Thus, it is necessary to conduct further studies for understanding postnatal learning process deeply.

Overall, previous studies show that prenatal or postnatal exposure of flavor influenced feed preference of young animals. Phytogenic feed additives are good sources of volatile aroma compounds including variety essential oils (Máthé, 2009). Therefore, supplementation of phytogenic feed additives which have certain volatile compounds may improve voluntary feed intake by prenatal or postnatal learning through their maternal diet.

2. Phytogenic feed additives

2.1 General aspects of phytogenic feed additives

Phytogenic feed additives attracted increasing interest as an alternative feed additive which can replace antibiotics in animal diets (Windisch et al., 2008; NRC, 2012). Phytogenic feed additives include herbs, spices, essential oils, which are extracts derived from plant materials (Steiner, 2009; Jacela et al., 2010). The most common phytogenic substances used in diets fed to swine are oregano, thymol, carvacrol, and garlic (NRC, 2012). The mode of action for most phytogenic feed additives is not clear in vivo situation. But, several researches explained and demonstrated phytogenic feed additives may affect performance of animal (Windisch et al., 2008; Jacela et al., 2010; Wang et al., 2011; NRC, 2012). According to the review of Máthé (2009), the advantages of phytogenic feed additives in animal feeds may help to;

- Reduced risk of enteric imbalances (such as diarrhea), especially of young animals
- Improved growth performance, including weight gain and feed conversion
- Stimulated feed intake
- Increase in egg production
- Reduction of mortality
- Better acceptance of feed ingredients with unpleasant taste
  (such as rapeseed byproducts, protein-concentrates or mineral premixes)
- Reduced need to apply of chemotherapeuticals
- Improved product quality (in terms of taste, color or texture)
- Improved barn-climate including the reduction of unpleasant odor and toxic gases
- No withdrawal period in most cases
- Presumably no harmful residues in animal products

2.2 Antioxidant effects

Increasing oxygen radical generation and lipid peroxidation have been suggested to be harmful of a wide range of compounds (Halliwell and Gutteridge, 1985). Mahan et al. (2007) reported that antioxidant nutrients (selenium, glutathione peroxidase, ascorbate, and α-tocopherol) in serum declined sharply during late gestation and lactation which are periods of high stress. These lower antioxidant status influenced tissue damage and pregnancy complications (Mahan et al., 2007; Sugino et al., 2007). Various studies have been conducted to prevent toxic results from oxygen radical (Sies, 1997). And antioxidative properties of plant extracts are well described (Cuppett and Hall, 1998; Wei and Shibamoto, 2007; Tan et al., 2015). Many active components of plant extracts can prevent lipid peroxidation through removal free radicals or activation of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase (Frankic et al., 2009).

Among a variety of plants extracts have antioxidative constituents, the volatile oils from the Labiatae family (e.g. basil, mint, oregano, rosemary, sage, and thyme) have high ability as antioxidant agent (Economou et al., 1991; Cuppett and Hall, 1998; Windisch et al., 2008). Janz et al. (2007) reported that oregano-fed (0.05% supplementation to finisher feed) pork showed a tendency towards reduction of lipid oxidation. Wei and Shibamoto (2007) observed that parsley seed, ylang-ylang, jasmine oil, and rose inhibited hexanal oxidation by nearly 100% over 40 days, it was similar result with α-tocopherol which was well known as antioxidants. And according to the study of Tan et al. (2015), supplementation of oregano essential oil to sow diet reduced oxidative stress during delivery and improved performance of their piglets from this mechanism. Also, several studies explored potential capacity of anise or anethole as a natural antioxidants in vitro essay (Freire et al., 2005; Aly et al., 2014; Kanatt et al., 2014; Sá et al., 2017). However, results were inconsistent by extraction procedures and researchers (Padmashree et al., 2007).

2.3 Antimicrobial effects

To find alternative sources which can replace the antibiotics as growth

promoters is very important in animal industry. Antibiotics have been used for over 40 years in commercial farm for treating identified illness, preventing illness advance and increasing growth performance of animals (Pluske, 2013).

Plant extracts and essential oil from plants are well known about inhibiting effects of pathogens in vitro (Smith-Palmer et al, 1998; Hammer et al., 1999; Dorman and Deans, 2000; Si et al., 2006; Windisch et al., 2008). However, several studies showed that the wide variation in the antibiotics properties of plant extracts (Smith-Palmer et al., 1998; Dorman and Deans, 2000; Si et al., 2006). Despite wide variation in the antibiotics properties by sources, the components with phenolic structures reported highly active against microorganisms (Dorman and Deans, 2000). Carvacrol, eugenol and thymol are typical essential oils which have phenolic structure. Similar results were reported that lemongrass, oregano and bay inhibited all organisms (Hammer et al., 1999) and bay, cinnamon, clove and thyme inhibited bacteriostatic concentration of 0.075% (Smith-Palmer et al., 1998). Also plant extract combination of oregano, anise and citrus peels reduced contents of anaerobic and aerobic germs in chyme of ileum, caecum and colon (Kroismayr et al., 2008).

As a result, certain phytogenic feed additives may perform a role as non-antibiotic growth promoters in livestock and food preservatives, such as organic acids and probiotics (Smith-Palmer et al., 1998; Windisch et al., 2008).

3. Plant extracts from star anise (*Illicium verum*)

3.1 Feature of star anise extracts

Star anise (SA) is an aromatic evergreen tree has purple-red flowers and anisic fragrant star-shaped fruit (Wang et al., 2011). The fruit of Star has been used long time in traditional medicine and food industry with various actions; antimicrobial, antioxidant, insecticidal, analgesic, sedative and convulsive activities (Wang et al., 2011). Li and Liu (2000) reported that the essential oil from SA seed mainly includes anethole (70-94%), pinene, and α-terpineol. The major efficacy agent is anethole which is major component of essential oil of SA.

The biological property of anise oil is known that anise oil simulating the secretion of digestive enzymes and stimulating appetite (Wang et al., 2015). Additionally, anise supplementation showed similar effects like as phytoestrogen substances (Wang et al., 2015). The research with multiparous sow and piglets, anise supplementation increased concentrations of insulin-like growth factor-1 (IGF-1) in sow milk and prolactin in serum of sows, and therefore improved weaning weight of piglets (Wang et al., 2015). Similar results were also reported by Lei et al. (2015) that sow diets with anise increased ADFI of lactation sows and decreased backfat loss of lactation sow during lactation period. Expression of the IGF system controlled by estrogen and phytoestrogen (Liu et al., 1999; Ren et al., 2001).

Several studies explored potential capacity of SA as a natural antioxidant (Freire et al., 2005; Aly et al., 2014; Kanatt et al., 2014; Sá et al., 2017). According to the study of Padmashree et al. (2007), anise powder and ethanol/ water extract shown higher antioxygenic activities. And in vitro trial with goat preantral follicles, the addition of anethole to the culture medium was improve the development of follicles by reducing concentrations of reactive oxygen species (ROS) and increasing the percentage of oocytes able to resume meiosis (Sá et al., 2017). These results suggest that anise may have some antioxidant effects in animals. However, in vivo trials are very few that demonstrated the effects of anise as a natural antioxidant.

3.2 Anise flavor and pre- or postnatal learning

Anise oil and trans-anethole which is major component of anise essential oil have used prenatal and postnatal learning researches with pigs. Langendijk, et al. (2007) studied the effects of pre-and postnatal exposure to garlic and aniseed flavor on pre-and post-weaning feed intake in pigs. This study shown that early exposure of garlic and aniseed flavors in maternal diet increased later acceptance and improved adaptation during post weaning period. Figure 3 showed that late weaned (week 6) litters received the flavor feed had a higher feed intake from 3 to 10 days after weaning (Langendijk, et al., 2007).

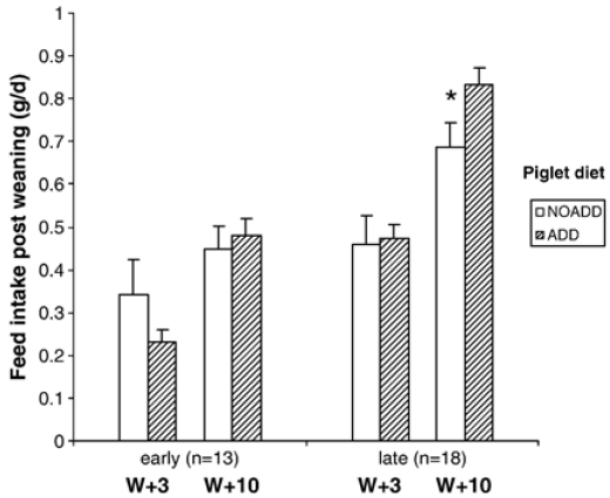

Figure 3. Effect of addition of garlic/aniseed to the post-weaning diet on feed intake of piglets at 3 days (W+3) and 10 days (W+10) post weaning. Piglets were weaned 'early' (4 weeks) or 'late' (6 weeks) (Langendijk, et al., 2007).
* Indicates significant difference between ADD and NOADD.

Oostindjer et al. (2009, 2010, and 2011) conducted several experiments to find prenatal and postnatal learning process and their impacts on behavior and stress level of piglets. Oostindjer et al. (2009) reported that prenatal anise flavor exposure influenced behaviors of piglets due to stress levels from re-exposure. Also, Oostindjer et al. (2010) observed similar results that prenatal flavor exposure enhanced feed intake and body weight and reduced stress related behaviors and diarrhea piglets after

weaning. Stress relationship with prenatal exposure of anise flavor and re-exposure after weaning demonstrated by Oostindjer et al. (2011). According to the experimental results of this research, flavor-exposed piglets tended to show a faster decrease in salivary cortisol levels after weaning. Initial stress response was similar for all piglets however the familiar flavor influenced to recover quickly.

Blavi et al. (2016) reported that anethole, cinnamaldehyde, and eugenol were detected from amniotic fluid of sows fed diet with flavored additives (containing >25% anethole and cinnamaldehyde and >10% eugenol) from 73 days to farrowing (Figure 4). In this study show that amniotic fluid can be the prenatal learning pathway of flavor from maternal diet to their progeny during gestation period.

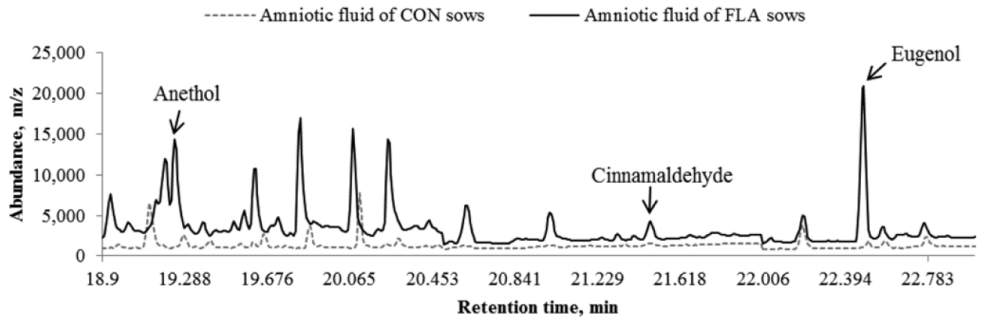

Figure 4. Presence of anethole, cinnamaldehyde, and eugenol in amniotic fluid. The solid line represents the mean of amniotic fluid samples of the flavored (FLA sows = sows received flavored feed.) sows and the dotted line represents the mean of amniotic fluid samples of the control (CON sows =sows were offered a non-flavored feed) sows (Blavi et al., 2016)

Many studies with anise flavor in sow and piglets diets showed that prenatal and postnatal exposure of anise flavor may have positive effects on feed intake, growth and behavior of piglets during post-weaning period (Langendijk, et al.,2007; Oostindjer et al., 2009, 2010, 2011; Blavi et al., 2016).

However, there is no evidence of growth and reproduction performance effects of prenatal and postnatal exposure of SA supplementation in maternal diets and progeny diets. Similarly, there is no information about the effect of imprinting

and SA supplementation during growing-finishing period on performance and meat quality of growing-finishing pigs.

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Chapter III. Effects of Dietary Star Anise (*Illicium verum*) Supplementation during Late Gestation and Lactation on the Performance of Multiparous Sows and Their Progeny until 21 Days Post-weaning

ABSTRACT: This study was conducted to investigate the effects of star anise (SA) supplementation during late gestation and lactation on the performance of multiparous sows and their progeny until 21 d post-weaning. A total of 40 pregnant sows [Yorkshire × Landrace] were housed in an individual stall and allocated on the basis of body weight (BW), backfat thickness (BFT) and parity in a completely randomized design (CRD) with one of two treatments, either 0% or 0.1% SA supplementation. After lactation period, a total of 160 weaning pigs were used to investigate imprinting effect of SA on growth performance and antioxidant status. Dietary treatments were equally allocated as their litter. In late gestation, sows fed SA showed significantly higher serum total antioxidant status (TAS) activity (P=0.03) and a tendency of increased lactation average daily feed intake (P=0.08). When sows were fed SA during late gestation tended to increase litter weight at 21 d of lactation as well as significantly increase litter weight gain (P=0.02, P=0.04, respectively). Sows consumed SA in late gestation tended to have lower protein in sow milk (P=0.07). In contrast, supplying SA in gestation diet resulted in increasing lactose content in the milk (P<0.01). Gestation × lactation effect was noted in the total solid and free fatty acid (FFA) concentration of sow milk at 21d lactation (P=0.06, P=0.04, respectively). At weaning, supplying SA diet during late gestation reduced concentration of serum cortisol in piglet and epinephrine at weaning (P=0.01, P=0.04, respectively). Moreover, there was a gestation × lactation interaction in serum cortisol concentration of piglet (P=0.01). Supplementing SA during late gestation tended to enhance gain to feed (G:F) ratio of weaning pigs at one and two weeks (P=0.08). Similarly, supplementing SA during lactation increased gain to feed ratio both in two and three weeks and overall period (P=0.03, P=0.05, respectively). The SA

supplementation during lactation reduced serum cortisol concentration of weaning pigs in initial ($P=0.05$). Main effects of gestation, lactation, and gestation × lactation interactions were noted in serum superoxide dismutase (SOD) activity at initial and three week of weaning pigs ($P<0.01$, respectively). Therefore, our results suggested that inclusion of SA in gestation and lactation diet showed higher serum antioxidant properties in sows and increased lactose and free fatty acid in sow milk, consequently enhancing litter weight and feed efficiency at 21 d post-weaning.

Key words: Antioxidant, Imprinting, Piglet, Sow, Star anise

1. INTRODUCTION

Piglet weaning is the most critical event in the pork production cycle due to adaptation and stress in response to the simultaneous stressors imposed on pigs at weaning (Bruininx et al., 2002). Multiple stressors during weaning include changing diet form and environment, mixing of non-littermates, lack of appropriate stimuli, separation from sow, as well as weaning at an age much earlier than natural (Gardener et al., 2001).

To cope with these problematic consequences, flavor imprinting in young animals diets are of interests since majority of antibiotics have been prohibited (Zhong et al., 2011). Numerous studies have proved that the prenatal exposure to certain flavors, derived from the maternal diet, modulate the food preferences and neophobias of young animals in various species (Molina et al., 1995; Schaal et al., 1995; Semke et al., 1995). In these species, the introduction of flavor into the amnion either by direct infusion or maternal ingestion affects later infantile responses to the same stimuli (Schaal et al., 2000). Star anise (*Illicium verum*, SA), an herbaceous annual plant, contains essential oils. The major component of its volatile oil is anethole, which accounts for 80-90 % (Nahar et al., 2012). In swine, several studies indicated that anise flavor to lactating sows enhanced IGF-1 concentration in the sow milk, prolactin in serum of sows and higher milk yield (Wang et al., 2015). For piglets, it reduced stress related behavior (Oostindjer et al., 2009), increased post-weaning feed intake (Oostindjer et al., 2011), and higher ADG (Wang et al., 2015).

However, several scientific researches are mostly focused on the effect of dietary SA supplementation in sow diet on the growth performance of their litters during lactation, not sequential effect in post-weaning period. The hypothesis tested was that inclusion SA during late gestation and lactation to sow would increase the feed intake and antioxidant status containing the same flavors during lactation of piglets, thereby consecutively affect positively on the reduction of weaning stress and enhancement of growth performance after weaning.

2. MATERIALS AND METHODS

All experimental procedure performed in this study was approved by the Institutional Animal Care and Use Committee of the Seoul National University. Two trials were conducted with pigs in a multisite production system. The sow experiment was conducted at the Jacob Swine Research Farm, located in Eumseong-gun, Chungcheongbuk-do, Korea Republic. The weaning pig experiment was conducted at the Dae-woo Swine Research Farm, located in Muan-gun, Jeollanam-do, Korea Republic. The distance between two locations is about 320.6 km and takes 4 hours to drive.

The plant extract using in this study named Revest® Arom 1033 Anise P (BIOMIN Phytogenics GmbH, Stadtoldendorf, Germany), which involved 15.0% of natural flavor extracts from star anise (*Illicium verum*). The 85.0% of this product composed with binder and carrier which were silica dioxide and sodium chloride.

2.1 Procedure

A total of 40 multiparous gestating sows [Yorkshire x Landrace] were used in a 2 x 2 factorial design experiment. The factors were supplementation of SA either in late gestation and lactation period, respectively. An overview of all experimental treatments and procedures was given in Figure 1. Experimental sows were introduced in an individual gestation stall in an environmentally controlled barn. Estrus was diagnosed twice daily in the presence of a mature boar, using the backfat pressure test. Sows were twice served artificial insemination (AI) with fresh diluted semen (Darby A.I. center, Chungju-si, Chungcheongbuk-do, Korea Republic) at 12 hour intervals. Pregnancy of the gilts was diagnosed by an ultrasound analyzer (Easyscan, Dong-jin BLS Co., Ltd., Gwangju-si, Gyeonggi-do, Korea Republic) on d 28 and 35 postcoitum.

On day 90 of gestation, the sows were allocated on the basis of body weight (BW), backfat thickness (BFT) and parity in a completely randomized design (CRD) to one of two treatments, either 0% or 0.1% SA. On day 110 of gestation, sows were

introduced into individual farrowing crates (2.2 m × 1.5 m). Also, daily feed intake was gradually reduced by 0.2 kg/day for each sow until the day of delivery.

Within 24 h post-farrowing, sows in each treatment group were assigned randomly to corn-and soybean meal-based lactation diets supplemented with either 0% or 0.1% SA. The lactation diet was gradually increased from 1.0 kg/d by 1.0 kg/d until 5 d postpartum with a free access to water. For piglets, procedures including Fe-dextran (150 ppm) injection, ear notching, needle teeth clipping and tail docking were practiced. Additionally, piglets were cross-fostered across treatments within 3 d after birth to balance suckling intensity across sows with equalization of litter size, and thus to minimize any impact of initial litter size potentially affecting litter growth.

After weaning, pigs were moved into another site. A total of 160 weaning pigs ([Yorkshire×Landrace]×Duroc, 7.37±0.98 kg) were used to investigate the consecutive imprinting effect of SA on weaning pigs' growth performance as well as antioxidant status. Ten piglets per litter were selected and allocated same treatment as their mother. All weaning pigs were top-dressed same amount of SA (0.05%) in the corn-soybean meal based diet until d 21 post-weaning.

2.2 Housing

The experimental sows were housed in a gestation barn with an individual crate (2.15 m × 0.6 m) with a fully slatted concrete floor. Room temperatures and ventilation rates were measured and determined with sensors, which were installed near the sows and were manipulated by an automatic climate control system (KO-850, KUN OK Co., Ltd., Nonsan-si, Chungcheongnam-do, Korea Republic). The average temperature during the entire experimental period was 20.0°C. Lights were provided with three fluorescent lights and several windows. Feed was accurately weighed by a scale (SW-1W, CAS Co. Ltd., Yangju-si, Gyeonggi-do, Korea Republic), and was provided twice a day (08:00 and 16:00) by feed buckets through an individual feeder with one waterer per sow.

At d 110 of gestation, all sows were moved to the farrowing crates (2.20 m × 0.65 m) with partition walls (2.50 m × 1.80 m) after washing and disinfecting their

body. During lactation, the room temperature and air conditioning of the farrowing barn were kept automatically at 25 ± 3℃by heating lamps and ventilation fans. After weaning, sows were moved to the breeding barn again for the next conception.

After weaning, 8 pigs were housed in a 1.2 m × 3.6 m plastic floor, equipped with a feeder and a nipple drinker to allow freely access to feed and water during the three week experimental period. The ambient temperature in the weaning house was kept 31℃ during the first 7 days and lowered 1℃ every week to 28℃.

2.3 Experimental diets

The ingredient composition and calculated nutrient content of diets were shown in Table 1. Star anise 0.1% was top-dressed on both gestation and lactation control diet. Gestation diet contained 3,075 ME kcal/kg and 13.0% of crude protein (CP), 0.79% of lysine, 0.49% of methionine+cysteine, 0.57% of threonine, and 0.15% of tryptophan, respectively. Lactation diet contained 3,116 ME kcal/kg and 17.7% of CP, 1.02% of lysine, 0.63% of methionine+cysteine, 0.66% of threonine, and 0.22% of tryptophan, respectively. Gestation diets were provided daily at 2.0, 2.2, 2.4 kg/day for the first, second and over third parity, respectively. However, lactation and weaner diet was provided *ad libitum* and water was available freely in both periods. Weaning pig's diets contained 3,382 ME kcal/kg and 19.89% of CP, 1.56% of lysine, 0.89% of methionine+cysteine, 0.96% of threonine, and 0.26% of tryptophan, respectively. 0.05% of SA was equally top-dressed for all treatment. All other nutrients were met or exceeded requirements of NRC (2012).

2.4 Measurements and sample collection

In the gestation barn, BW and BFT of sows were measured at days 90, and 110 of gestation and 12 h, 21 d postpartum. BFT was measured at the P_2 position (last rib, 65 mm from the center line of the back) on both sides of the back bone using an electric measuring device (Lean-Meater®, Renco Corp., Minneapolis, MN, USA). Values from the two measurements were averaged to record a single BFT. Litter traits included the number of piglets born alive, stillborn, mummies, and losses. Within 24

h after birth, the litters were weighed individually by scale (SW-1W, CAS Co. Ltd., Yangju-si, Gyeonggi-do, Korea Republic). Litter and mean pig birth BW, weaning BW, and mean BW gain from birth-to-weaning were calculated. To observe piglet uniformity, coefficient of variation (CV), standard deviation (SD) was calculated at piglet birth and weaning BW. All sows were moved to gestation stalls as soon as all piglets were weaned (approximately 21 days), and then weaning-to-estrus interval (WEI) was scored. Body weight and feed consumption of weaning pigs were recorded at d 0, 3, 7, 14 and 21 post-weaning to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F ratio).

Sow blood collection was taken by venipuncture of the jugular vein using 10 ml disposable syringes at the same time of measuring the BW and BFT. Piglet (n=4) blood was collected from the anterior vena cava using 3 ml disposable syringes at 12 h, 21 d postpartum and 5 ml disposable syringes within one hour after weaning. Weaning pig (n=4 for each treatment) blood was collected from the anterior vena cava using 5 ml disposable syringes at initial, 1 week, 2 week, 3 week after 3 hours fasting.

All samples were enclosed into serum tube (SST^{TM} Ⅱ Advance, BD Vacutainer, Becton Dickinson, Plymouth, UK) as well as ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer K_2E, Becton Dickinson, Plymouth, UK) and centrifuged at 3000 rpm and 4 °C for 5 min after clotting at room temperature for 30 min (5810R, Eppendorf, Hamburg, Germany). The upper liquid (serum) of the blood was separated to a microtube (Axygen, Union City, CA, USA) and stored at −20 ℃ until later analysis.

Colostrum samples were taken from functional mammary glands of each treatment within 24 h postpartum, whereas milk samples were taken at 21 d postpartum. Colostrum and milk were collected from the first and second teats after an intravascular injection with 5 IU oxytocin (Komi oxytocin inj., Komipharm International Co., Ltd., Siheung-si, Gyeonggi-do, Korea Republic) in the ear. After collection, samples were stored in a freezer (−20 ℃) until further analysis. Proximate analysis of colostrum and milk was determined using a Milkoscan FT 120 (FOSS,

Hillerod, Denmark).

2.5 Chemical analysis

The serum superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in sow serum were measured using commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. Hemoglobin concentration (for estimation of SOD and GPx activities) was measured in a microplate reader (Versamax, Molecular device, USA). Total antioxidant status (TAS) was measured using an automated method (Erel, 2004). Plasma for cortisol concentration was measured using a commercially available ELISA kit (swine cortisol ELISA kit, Endocrine Technologies, U.S.). Plasma epinephrine and norepinephrine were assayed using an ion-exchange purification procedure followed by liquid chromatography with electrochemical detection (Hay and Mormède, 1997). Briefly, the samples were loaded onto cationic columns, and the catecholamines were eluted with boric acid. The eluates were assayed via HPLC with electrochemical detection with an oxidizing potential of +0.65V.

2.6 Statistical analysis

Sow data was analyzed as a complete randomized design with SA supplementation (0 or 0.1%) in gestation and lactation diet. Performances of SA supplementation in late gestation including physiological response and serum oxidative status were analyzed via pairwise T-test, lactation performance including physiological response, reproductive performance, as well as blood and milk analysis were analyzed via two-way analysis of variance. Individual sow was considered as the experimental unit.

Data of weaning pig including growth performance and blood analysis was analyzed as a randomized complete block design with two-way ANOVA. The pen of pigs was used as the experimental unit in growth performance, and individual piglet was used as the experimental unit in blood profiles. The significant difference was set

at $P < 0.05$, and tendencies were determined if $0.05 \leq P < 0.10$. All the data was analyzed by the General Linear Model (GLM) procedure of SAS (version 9.4: SAS Institute Inc., Cary, NC, USA).

3. RESULTS

In late gestation, there was no significant difference in physiological response in relation to the effect of supplementing SA (Table 3). However, TAS activity at d 110 was significantly higher (P=0.03) in Anise treatment (Table 4).

Neither the reproductive performance nor piglet uniformity was affected by SA supplementation (Table 5). Moreover, no gestation × lactation diet interaction was observed.

Despite there were no statistical significance in physiological response and average daily feed intake by SA supplementation, sows fed SA in late gestation tended to increase backfat thickness within 24 hrs parturition (Gestation, P=0.07). Likewise, sows consumed SA in gestation tended to have much loss in backfat during lactation period and in lactation tended to have less loss in backfat during lactation period (Gestation, P=0.10; Lactation, P=0.06; Table 6). There was a tendency of increased lactation average daily feed intake (ADFI) in the sows fed SA in late gestation (Gestation, P=0.08).

Sows fed SA during lactation increased number of weaning pigs (Lactation, P=0.02; Table 7). When sows were fed SA during late gestation, litter weight at 21 d of lactation tended to increase as well as significantly increase litter weight gain (Gestation, P=0.06, P=0.04, respectively).

The effects of SA supplementation on the serum oxidative status was represented in Table 8. Sows consumed SA in late gestation tended to have lower TAS activity at 21d lactation (Gestation, P=0.09). In piglets at 21 d lactation, a reduced serum GPx activity was observed in the both treatments, which sows fed SA both in gestation (P=0.02) and lactation (P<0.01), respectively.

The milk composition of lactating sows fed diets with or without

supplemental SA was shown in Table 9. The casein and milk fat of colostrum and ordinary milk were not affected by dietary treatment. On the other hand, sows consumed SA in late gestation tended to have lower protein in sow milk (Gestation, P=0.07). In contrast, supplying SA in gestation diet resulted in increasing lactose content in the milk (Gestation, P=0.01). Solid not fat and density of the sow milk tended to decreased as SA supplemented in the lactation diet (Lactation, P=0.08). Gestation × lactation effect was noted in the total solid and free fatty acid (FFA) concentration of sow milk at 21d lactation (GxL, P=0.06, P=0.04).

Supplying SA diet during late gestation reduced concentration of piglet serum cortisol (Gestation, P=0.01, Table 10) and epinephrine at weaning (Gestation, P=0.04, Table 10). Furthermore, there was a gestation × lactation interaction in the piglet serum cortisol concentration (GxL, P=0.01).

The effects of SA supplementation during late gestation or lactation diet on the growth performance of weaning pigs were presented in Table 11. There were no significant effects observed on BW, ADG, and ADFI. However, supplementing SA during late gestation tended to enhance gain to feed ratio of weaning pigs at one and two weeks (Gestation, P=0.08). Likewise, supplementing SA during lactation increased G:F ratio both in two and three weeks and overall period (Lactation, P=0.03, P=0.05).

The effects of SA supplementation during late gestation or lactation diet on the blood analysis of weaning pigs were represented in Table 12. The SA supplementation during lactation reduced serum cortisol concentration of weaning pigs in initial (Lactation, P=0.05). Additionally, tendency on gestation × lactation in this stage was observed (GxL, P=0.06). Main effects of gestation, lactation, and gestation × lactation interactions were noted in serum superoxide dismutase (SOD) activity at initial and three week of weaning pigs (P<0.01, respectively).

4. DISCUSSION

The current experiment investigated the effects of supplementing SA in the sow diet during late gestation and lactation period on physiological response and reproductive performance, as well as the subsequent growth and health in the post-weaning period of their progeny. Results from our study indicated that supplementation of SA to the gestation diet increased serum antioxidant status, whereas to the lactation diet improved milk quality of sows, which affected the overall improvement of piglet growth performance. Moreover, in post-weaning period, pigs with postnatal anise exposure decreased stress related hormones at weaning, resulting enhanced feed efficiency and serum antioxidant status (SOD value).

It has been known that the placenta is the major source of oxidative stress (Kaya et al., 2013). The oxidative stress occurs when cellular reactive oxygen species and free radicals overwhelm the antioxidant defense mechanisms, resulting in macromolecular damage to proteins, lipids and DNA (Valko et al., 2007). The oxidative stress elevated as the gestational week increases, which can cause a variety of pregnancy complications such as preterm labor, fetal growth restriction, preeclampsia and miscarriage (Gupta et al., 2005; Sugino et al., 2007). Anethole, extract from SA, belongs to a family of chemicals called phenolic compounds. Although the mechanism behind these effects is not clear, several studies noted that there is highly significant linear correlation between total antioxidant capacity and phenolic content, owing to the effectiveness of those phenolic species as good scavenges of hydrogen peroxide, superoxide, and free radicals (Velioglu et al., 1998; Dorman et al., 2003; Cai et al., 2004). Evidence can also be found in the studies of Wang et al. (2015) that sows fed 0.5% of SA in the late gestation to lactation diet enhanced serum antioxidant status of sows. In agreement, our data confirmed the action of SA as an antioxidant, which would promote the repair and maintenance of the damage membranes, being able to contribute to protection from oxidative damage in late gestation period.

Current experiment indicated that sow average daily feed intake (ADFI) during lactation was positively affected by dietary SA supplementation. This is consistent with reports that supplying SA in sow diet during lactation increased 4% of voluntary feed intake compared to control diet (Wang et al., 2015). In case of broilers, there was a significantly improved ADFI on 0.1% SA supplemented treatment (Al-Kassie, 2008). Based on the result of these studies, it can be assumed that the beneficial effect of SA inclusion in sow diet may primarily play a key role as an appetizer, preventing excessive backfat loss during lactation as well as providing piglets more nutritive value of milk.

Litter weight gain and number of pigs weaned are known to be associated with milk production or nutrient concentration in milk (Noblet and Etienne, 1987; King et al., 1993). Increased piglet and litter weight gain in SA supplemented sows might be indicative of increased milk or increased nutrient concentrations in milk. SA has been used for millennia to increase milk secretion in humans and other animals, thereby categorizing as galactagogue substance (Gabay, 2002). This property explained that their pharmacologic effects through interactions with dopamine receptors, inhibiting the secretion of the milk-producing hormone (Badgujar et al., 2014). Anethole might influence milk secretion by competing with dopamine at the appropriate receptor sites, thereby inhibiting the anti-secretory action of dopamine on prolactin (Albert-Puleo, 1980). Wang et al. (2015) indicated that supplementation of SA during lactation improved weaning weight of piglets, and milk yield, mainly due to increased concentrations of insulin-like growth factor-1 (IGF-1) in sow milk and prolactin in serum of sows. Although our data did not indicate hormonal analysis, it can be assumed that increased nutrient concentrations of lactose in sow milk by SA supplementation resulted in amelioration of litter weight gain and number of weaning pigs. This is in line with the studies of Matysiak et al. (2012) that plant extract mainly consisting of carvacrol, cinnamaldehyde and capsicum oleoresin in the lactation sow diet increased the concentration of lactose in the milk, suggesting that it can prevent hypoglycaemia and reduce piglet mortality. One possible mechanism for the SA supplementation with increased concentration of lactose in the sow milk can be

explained by the research of Kreydiyyeh et al. (2003) that anise seed oil significantly enhanced glucose absorption in the jejunum by increasing the Na+-K+ ATPase which is expected to increase the sodium gradient. This may lead to stimulate the dynamics of glucose uptake by the porcine mammary gland for milk production, increasing milk lactose and fatty acid concentration. There is sufficient experimental evidences to support the concept that plasma glucose contribution to milk fatty acid synthesis is 37%, whereas contribution to lactose is 60 to 70%, mainly due to inverse proportion of insulin concentration during lactation (Spincer and Rook, 1969; Reynolds and Rook, 1977).

Activities of serum antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) were determined in the present study. These intracellular antioxidant enzymes constitute the primary antioxidant defense system against reactive oxygen species (ROS) therefore they are regarded as sensitive markers of oxidative stress (Stukelj et al., 2013). Both increased and decreased levels have been reported in different diseases as a consequence of enhanced ROS production either by up-regulation of enzyme activity or utilization of the antioxidant enzymes to counter the ROS (Mates, 2000; Valko et al. 2007). In the current experiment, we found no effects on serum antioxidant status in sows at 21 d of lactation. Unlike what we expected, however, there is a reduced serum GPx activity in piglets at 21 d of lactation in supplementation of SA. We assumed that it can be a consequence of in-built compensatory mechanism (Kataria and Kataria, 2012) that deserve to be further investigated.

Inclusion of SA in the sow diet during late gestation reduced piglet serum cortisol and epinephrine concentration at weaning. Moreover, we found gestation×lactation interaction in serum cortisol level. This result was similar finding with Oostindjer et al., 2011. They reported that re-exposure of familiar flavor after imprinting during pre- or postnatal period reduced salivary cortisol level of piglets. And also inclusion of SA in the sow diet during lactation reduced piglet serum cortisol at initial of post-weaning experiment. Additionally, this result shown that gestation×lactation interaction in serum cortisol level at initial. This result is

noteworthy to mention that blood samples were collected right after 4 hour drive to multisite research farm plus 1 hour of pen allocation, which is extremely stressful condition for weaning pigs. When the piglets are shipped to another site to continue their growth process, the level of stress they suffer increases, and the physiological, metabolic and behavioral repercussions become more severe. In this vein, several studies have noted that transportation of weaning pigs can influence on elevation of serum cortisol level and creatine phosphokinase (CPK), which are associated in response to stressful stimuli (Bradshaw et al., 1996; Hicks et al., 1998) and have shown to cause behavioral changes (Hicks et al. 1998), fatigue (Lambooy, 1988) and weight loss (0.4 ± 0.1 kg; $P < 0.01$) regardless of trip duration (Wamnes et al., 2006). In line with, the beneficial effects of adding the flavor in sows' diet may indicate indirect effects on anti-stress of piglets at weaning. Possible explanation can be found in the studies of Karimzadeh et al. (2012). They found that production of dark neurons in the rat brain, produced in stressful conditions such as acute physical stress, normal ageing process in cerebellum and postmortem, cause disturbance in ion gradient (Na/K ATPase pump) and increases excitatory neurotransmitters like glutamate, can be significantly inhibited by anise oil supplementation. Considering conditions of current experiment, piglets indirectly exposed and/or consumed SA from their dams' diet prevented production of dark neurons by inhibition of seizure attacks and, therefore, may act as a neuroprotective substance, resulting diminution of weaning stress. Similar result has been found in other mammals that neonates recognize the flavor not only via inhalation and ingestion of amniotic fluid but also through extracorporeal secretions such as saliva, body odors, feces, or urine (Becques et al., 2009).

In placental mammals, phenolic flavors such as anethole can be transmitted to amniotic fluid and/or placental blood stream, thereby the fetus can perceive olfactory and gustatory preferences, which is called maternal imprinting (Oostindjer et al., 2009). Several studies have been investigating to evaluate the imprinting effect of various phenolic flavors with pigs for more than three decades. Langendijk et al. (2007) concluded that piglets born to a mother who had consumed garlic and aniseed

diet during antenatal and postnatal exposure had a higher feed intake of the odorized food than of the control diet. Similar result can be found in the study of Oostindjer et al. (2010) that it is more effective for piglets to exposure anise flavor a combination of pre- and postnatal period, not postnatal solely. More recently, the prenatal flavor in the diet affected feed preference of piglets after weaning, leading to increase feed intake, in turn, resulting in enhancement of growth (Blavi et al., 2016). In contrast, however, current experiment observed that the longer latency of piglets exposed to anise postnatally to explore the anise feeder showed no significance on feed intake, with the exception of enhanced feed efficiency in the last phase (2 to 3 week). The difference of feed preference owing to the imprinting impact may be explained in a number of ways. First, considering experimental environment, each treatment was not housed in individual farrowing barn. It may lead to outbreak cross-contamination among piglets, resulting in strong synergism at all treatment. Evidence can be found in similar experiments with human (Varendi et al., 1998) and rodents (Smotherman, 1982), explained that shared the same environment within the room could arise cross-contamination of volatile flavors. Second, appropriate level of SA inclusion in all the weaning pig diet may offset the imprinting effect on growth performance. However, the serum analysis data regarding to the anti-oxidant properties of weaning pigs in the current experiment support that the familiarity of the favor ameliorate in combination with anti-oxidative enzymes such as superoxide dismutase and stress reduction. It is generally believed that the most contributing factors affecting impairment of gastrointestinal integrity is oxidative stress, causing intestinal inflammation (Hu et al., 2014). Antioxidant enzymes are an important part of the antioxidant system protecting the cells against oxidative stress. According to the review article of Windisch et al. (2008), phytogenic substances positively impact on stimulation of digestive secretions and gut maturation, by enhancing anti-inflammatory and oxidant properties. In this study, there was a markedly higher activity of serum SOD in piglets fed SA from postnatal period, suggesting that SA could improve the anti-oxidative capability of weaning pigs, leading to increase intestinal integrity, in turn, resulting the advantage of weaning pigs' feed efficiency.

5. CONCLUSION

Supplementation of SA in the sow diet during late gestation and lactation enhanced oxidative status of sows as well as piglet growth. The increased litter weight gain was associated with increased concentrations of lactose and free fatty acid in milk at d 21 of lactation. For piglets, SA reduced weaning stress as well as showed potential possibility to enhance feed efficiency. It is therefore quite likely that SA could be considered a good source of feed flavor with significant antioxidant activities for sow diet. However, further studies are needed for evaluation of stress reduction mechanism.

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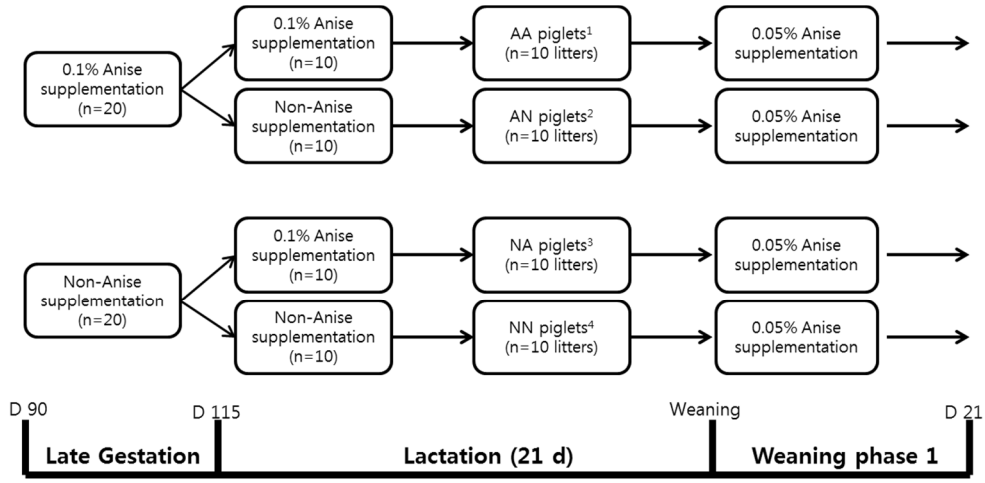

[1] piglets from gestation basal diet +0.1%SA, lactation basal diet +0.1%SA
[2] piglets from gestation basal diet +0.1%SA, lactation basal diet
[3] piglets from gestation basal diet, lactation basal diet +0.1%SA
[4] piglets from gestation basal diet, lactation basal diet

Figure 1. The experimental design and timeline of feeding regimen.

Table 1. The formulas and chemical composition of experimental diet (gestation and lactation)

Item[1]	Gestation		Lactation	
	Control	Anise	Control	Anise
Ingredient, %				
Corn, yellow	72.55	72.55	63.7	63.7
Soybean meal, 45% CP	14.75	14.75	27.2	27.2
Wheat mill run	6.0	6.0	2.8	2.8
Animal fat	2.6	2.6	2.8	2.8
Monodicalcium phosphate	1.8	1.8	1.5	1.5
Limestone	1.3	1.3	1.3	1.3
Lysine sulfate, 51%	0.29	0.29	0.1	0.1
Salt	0.3	0.3	0.3	0.3
Choline chloride, 50%	0.1	0.1	0.1	0.1
L-threonine, 98%	0.1	0.1	0.0	0.0
Anise extract	0.0	0.1	0.0	0.1
Vit. Mix.[2]	0.06	0.06	0.05	0.05
Min. Mix.[3]	0.15	0.15	0.15	0.15
Total	100.0	100.1	100.0	100.1
Chemical composition[4]				
ME, kcal/kg	3,075.0	3,075.0	3,116.4	3,116.4
Crude protein, %	13.0	13.0	17.7	17.7
Calcium, %	0.85	0.85	0.83	0.83
Phosphorus, %	0.71	0.71	0.67	0.67
Lysine, %	0.79	0.79	1.02	1.02
Methionine+cysteine, %	0.49	0.49	0.63	0.63
Threonine, %	0.57	0.57	0.66	0.66
Tryptophan, %	0.15	0.15	0.22	0.22

[1]Treatments: Gestation diets were fed 2.4kg/day in two separate meals; lactation diets were fed *ad libitum* up to weaning at 21days. Star anise diets were supplemented with 0.1% anise extract.

[2]Provided per kg of diet:

Gestation: vitamin A, 10,800IU; vitamin D_3, 2,400IU; vitamin E, 72IU; vitamin K, 3.6mg; vitamin B_2, 7.2mg; vitamin B_6, 4.8mg; vitamin B_{12}, 30μg; pantothenic acid, 24mg; biotin, 324μg; niacin, 48mg; folic acid 3.12mg; thiamine, 1.56mg.

Lactation: vitamin A, 9,000IU; vitamin D_3, 2,000IU; vitamin E, 60IU; vitamin K, 3mg; vitamin B_2, 6mg; vitamin B_6, 4mg; vitamin B_{12}, 25μg; pantothenic acid, 20mg; biotin, 270μg; niacin, 40mg; folic acid 2.6mg; thiamine, 1.3mg.

[3]Provided per kg of diet: Fe, 165mg; Mn, 60mg; Zn, 99mg; Cu, 7.5mg; Se, 450μg; I, 1mg.

[4]Calculated value.

Table 2. The formula and chemical composition of experimental weaner diet

Item	Control diet (Anise 0.05%)
Ingredient, %	
Corn, yellow	48.426
Soybean meal, 45% CP	15.00
Permeate SBM, 54% CP	5.000
Whey permeate	6.940
Lactose	5.000
Whey	2.000
Wheat mill run	3.000
Plasma protein	4.500
White fishmeal	2.500
Coconut fat	2.000
Vegetable oil	1.500
Monodicalcium phosphate	0.780
Limestone	0.500
Lysine sulfate, 51%	0.756
Salt	0.300
Choline chloride, 50%	0.139
L-threonine, 98%	0.160
Anise Extract	0.050
Organic acid	0.400
Enzyme mix	0.140
Probiotic	0.154
DL-methionine, 98%	0.187
Zinc oxide, 90%	0.313
Tryptophan, 10%	0.092
Yucca powder	0.013
Vit. Mix.[1]	0.100
Min. Mix.[2]	0.100
Total	100.05
Chemical composition[3]	
ME, kcal/kg	3,382.00
Crude protein, %	19.89
Calcium, %	0.63
Phosphorus, %	0.64
Lysine, %	1.56
Methionine+cysteine, %	0.89
Threonine, %	0.96
Tryptophan, %	0.26

[1]Provided per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000IU; vitamin E, 60IU; vitamin K, 3.5mg; vitamin B_2, 8mg; vitamin B_6, 2mg; vitamin B_{12}, 35µg; pantothenic acid, 25mg; biotin, 100µg; niacin, 50mg; folic acid 3.1mg; thiamine, 1.5mg.

[2]Provided per kg of diet: Fe, 100mg; Mn, 50mg; Zn, 50mg; Cu, 80mg; Se, 400µg; I, 1mg.

[3]Calculated values.

Table 3. Effect of star anise supplementation on changes in sow body weight and backfat thickness during gestation

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=20)	Anise (*n*=20)		
Body weight, kg				
90d gestation	254.2	256.9	4.95	0.79
110d gestation	267.5	274.5	5.04	0.49
BW gain(90-110d)	13.3	17.6	5.90	0.72
Backfat thickness, mm				
90d gestation	25.5	25.2	0.88	0.86
110d gestation	25.1	28.1	0.83	0.06
BF gain(90-110d)	-0.4	2.9	1.23	0.18

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.

Table 4. Effect of star anise supplementation on serum antioxidant enzymes of sow during gestation

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=20)	Anise (*n*=20)		
90 day				
GPx[3], nmol/min/mL	--433.655--			
TAS[4], mmol/L	--0.97--			
SOD[5], U/mL	--2.36--			
110 day				
GPx, nmol/min/mL	505.99	522.03	32.260	0.82
TAS, mmol/L	0.90	0.95	0.013	0.03
SOD, U/mL	3.84	3.40	0.383	0.60

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.
[3] Glutathione peroxidase.
[4] Total antioxidant status.
[5] Superoxide dismutase.

Table 5. Effect of star anise supplementation on reproductive performance of sows

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=20)	Anise (*n*=20)		
No. of piglets/litter				
Total born	13.95	13.01	0.665	0.48
Stillborn	0.86	0.59	0.141	0.29
Mummy	0.17	0.11	0.071	0.62
Born alive	12.93	12.32	0.616	0.65
Uniformity				
SD[3], g	322.9	307.20	16.81	0.52
CV[4], %	24.57	23.06	1.393	0.54

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.
[3] Standard deviation
[4] Coefficient of variation

Table 6. Effect of star anise supplementation on physiological changes and feed intake of sow during lactation

Gestation	Control		Anise			P-value[2]		
Lactation	**Control**	**Anise**	**Control**	**Anise**	**SEM[1]**	**G**	**L**	**G x L**
Body weight, kg								
24hrs postpartum	243.6	235.6	254.8	247.7	5.57	0.32	0.51	0.96
21d lactation	233.8	222.9	244.1	246.4	5.15	0.11	0.68	0.53
BW changes (0-21d)	-9.8	-12.7	-10.7	-1.3	2.89	0.38	0.58	0.30
Backfat thickness, mm								
24hrs postpartum	25.6	22.9	28.0	26.8	0.86	0.07	0.26	0.66
21d lactation	24.6	23.4	26.0	25.9	0.87	0.28	0.72	0.76
BF changes (0-21d)	-1.0	0.4	-2.0	-0.9	0.35	0.10	0.06	0.78
ADFI, kg	4.59	4.60	5.24	5.15	0.168	0.08	0.90	0.88

[1] Standard error of means.

[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.

Table 7. Effect of star anise supplementation on litter performance of sow during lactation

Gestation	Control		Anise			P-value[2]		
Lactation	Control	Anise	Control	Anise	SEM[1]	G	L	G x L
No. of piglets								
Initial (after-fostering)	11.5	11.6	11.3	11.6	0.14	0.81	0.57	0.74
Final (21d lactation)	9.6	10.9	10.1	11.2	0.25	0.38	0.02	0.86
Litter weight, kg								
24hr postpartum	16.33	15.91	16.68	15.63	0.354	0.96	0.32	0.67
21d lactation	48.11	56.58	59.18	59.43	1.904	0.06	0.24	0.26
Weight gain (0-21d)	31.78	40.67	42.50	43.80	1.801	0.04	0.14	0.27
Piglet weight, kg								
24hr postpartum	1.43	1.38	1.47	1.35	0.030	0.97	0.17	0.53
21d lactation	5.08	5.23	5.81	5.35	0.155	0.18	0.63	0.33
Weight gain (0-21d)	3.65	3.85	4.34	4.00	0.144	0.15	0.81	0.36

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.

Table 8. Effect of star anise supplementation on serum antioxidant enzyme in sows and piglets at d 21 lactation

Gestation	Control		Anise			P-value[2]		
Lactation	Control	Anise	Control	Anise	SEM[1]	G	L	G x L
Sow at 21d lactation								
GPx[3], nmol/min/mL	244.42	236.71	303.12	263.01	22.093	0.38	0.62	0.73
TAS[4], mmol/L	0.99	0.99	0.96	0.97	0.008	0.09	0.81	0.81
SOD[5], U/mL	3.36	3.16	3.59	3.55	0.306	0.65	0.85	0.91
Piglet at 21d lactation								
GPx, nmol/min/mL	522.45	429.58	471.35	350.88	19.578	0.02	<0.01	0.58
TAS, mmol/L	0.72	0.76	0.85	0.73	0.035	0.49	0.53	0.28
SOD, U/mL	4.99	5.02	5.01	4.89	0.151	0.87	0.88	0.83

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.
[3] Glutathione peroxidase.
[4] Total antioxidant status.
[5] Superoxide dismutase.

Table 9. Effect of star anise supplementation on milk composition in lactating sows

Gestation	Control		Anise			P-value[2]		
Lactation	**Control**	**Anise**	**Control**	**Anise**	**SEM[1]**	**G**	**L**	**G x L**
Casein, %								
Colostrum	7.24							
Milk (21d lactation)	4.67	4.40	4.39	4.46	0.056	0.31	0.40	0.14
Fat, %								
Colostrum	6.97							
Milk (21d lactation)	6.19	5.95	5.87	6.98	0.223	0.42	0.33	0.14
Protein, %								
Colostrum	9.10							
Milk (21d lactation)	5.29	4.99	4.87	4.91	0.071	0.07	0.33	0.20
Lactose, %								
Colostrum	4.42							
Milk (21d lactation)	6.24	6.10	6.40	6.32	0.041	0.01	0.12	0.64
Total solid, %								
Colostrum	22.68							
Milk (21d lactation)	19.35	18.45	18.65	19.74	0.260	0.56	0.84	0.06
Solid not fat, %								
Colostrum	13.90							
Milk (21d lactation)	11.50	11.27	11.36	11.17	0.059	0.29	0.08	0.86
Density								
Colostrum	1038.8							
Milk (21d lactation)	1039.0	1038.8	1039.8	1037.3	0.405	0.62	0.08	0.15
FFA[3], %								
Colostrum	5.14							
Milk (21d lactation)	8.24	5.14	7.78	8.20	0.488	0.12	0.12	0.04

[1] Standard error of means
[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.
[3] Free fatty acid.

Table 10. Effect of star anise supplementation in sow diet on serum stress status of piglet at the weaning

Gestation	Control		Anise			P-value[2]		
Lactation	**Control**	**Anise**	**Control**	**Anise**	**SEM[1]**	**G**	**L**	**G x L**
Cortisol, ㎍/dL	6.32	3.80	2.62	3.80	0.463	0.01	0.34	0.01
Epinephrine, pg/mL	68.15	43.80	26.67	25.75	7.402	0.04	0.35	0.39
Norepinephrine, pg/mL	40.12	23.87	32.25	34.47	4.091	0.87	0.42	0.29

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.

Table 11. Effect of star anise supplementation in sow diet and weaning diet on growth performance of weaning pigs

Gestation	Control		Anise			P-value[2]		
Lactation	**Control**	**Anise**	**Control**	**Anise**	**SEM[1]**	**G**	**L**	**G×L**
Body weight, kg								
Initial	7.36	7.36	7.37	7.37	0.214	-	-	-
1 week	8.98	8.51	8.63	8.54	0.700	0.81	0.67	0.77
2 week	12.15	11.05	11.66	11.38	0.361	0.92	0.44	0.64
3 week	16.01	14.96	15.73	15.20	0.420	0.98	0.45	0.80
Average daily gain, g								
0-1 week	231	164	180	167	13.5	0.45	0.22	0.40
1-2 week	454	362	433	405	16.3	0.77	0.13	0.40
2-3 week	552	559	581	547	11.8	0.77	0.64	0.49
Overall	412	362	398	373	11.3	0.95	0.18	0.65
Average daily feed intake, g								
0-1 week	226	192	191	187	8.5	0.27	0.31	0.42
1-2 week	516	464	486	459	16.9	0.65	0.31	0.75
2-3 week	816	722	808	733	23.5	0.97	0.13	0.85
Overall	519	459	495	459	14.3	0.70	0.14	0.70
Gain to feed ratio								
0-1 week	1.022	0.846	0.904	0.881	0.0417	0.71	0.37	0.49
1-2 week	0.875	0.778	0.893	0.885	0.1452	0.08	0.13	0.19
2-3 week	0.679	0.786	0.724	0.744	0.0141	0.32	0.03	0.05
Overall	0.794	0.791	0.803	0.808	0.0103	0.95	0.05	0.16

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.

Table 12. Effect of star anise supplementation in sow diet and weaning diet on blood profiles of weaning pigs

Gestation	Control		Anise			P-value[2]		
Lactation	**Control**	**Anise**	**Control**	**Anise**	**SEM[1]**	**G**	**L**	**G×L**
Cortisol, ng/mL								
Initial	11.52	6.52	7.61	7.42	0.666	0.23	0.05	0.06
1 week	11.34	9.45	14.71	6.25	1.937	0.98	0.31	0.51
2 week	5.83	6.82	4.85	7.63	0.538	0.94	0.15	0.49
3 week	6.67	7.13	4.67	5.98	0.511	0.14	0.40	0.68
SOD[3], U/mL								
Initial	6.90	6.07	6.31	10.74	0.422	<0.01	<0.01	<0.01
1 week	8.00	6.38	5.29	7.54	0.449	0.45	0.76	0.07
2 week	8.13	7.88	8.79	6.75	0.336	0.75	0.14	0.25
3 week	7.27	9.42	7.56	13.72	0.553	<0.01	<0.01	<0.01

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, L: SA 0.1% supplementation or not during lactation, G x L: interaction between SA supplementation during gestation and lactation.
[3] Superoxide dismutase.

Chapter IV.Effects of Star Anise (*Illicium verum*) Supplementation during Late Gestation and Post-weaning on Performance of Sow and Their Progeny

ABSTRACTS: The present study was conducted to investigate the effects of star anise (SA) supplementation during late gestation and post-weaning on the performance of multiparous sows and their progeny. A total of 50 pregnant sows [Yorkshire × Landrace] were housed in an individual stall and allocated on the basis of body weight (BW), backfat thickness (BFT) and parity in a completely randomized design (CRD). Sows were offered SA treatment diets with 0.1% SA or control diet during late gestation. After lactation period, a total of 120 weaning pigs were used to investigate imprinting effect on weaning pigs' growth performance. Piglets were fed treatments diet with 0.02 or 0.04% SA after weaning. In late gestation, there were no significant differences in physiological responses in relation to the effect of supplementing SA. There were no significant differences in number of piglets, litter weight change, and piglet weight during lactation period. When sows were fed control diet, high level fat contents and total solid in sow milk at 21d lactation were observed (P=0.01, respectively). Supplementing 0.04% SA during post-weaning tended to enhance ADG at two to four weeks (Weaning, P=0.09). Additionally, prenatal exposure during late gestation group showed lower ADFI at initial time to one week (Gestation, P=0.09). Supplementing 0.02% SA during post-weaning increased gain to feed ratio significantly at 0-1 week (Weaning, P=0.02). Prenatal exposure of SA improved the CV of piglets at weaning (Gestation, P=0.02) and 4 week after weaning (Gestation, P<0.01). Serum cortisol levels tended to be lower at 4 weeks after weaning for prenatal exposure piglets to control piglets (GxW, P<0.01). These results suggested that inclusion of SA in gestation and re-exposure through post-weaning diets enhanced uniformity of piglets as well as reduced stress level of piglets. And less than 0.02% SA supplementation in post-weaning diets recommended to observe imprinting impacts in weaning pigs.

Key words: Imprinting, Sow, Star anise, Stress, Weaning pig

1. INTRODUCTION

The transition from suckling milk to the solid feed is a challenge for piglets after weaning in commercial pig farm, which are usually weaned at a young age (Williams, 2003; Bolhuis et al., 2009). With this changing of feed physical form and several stressors from environmental change interrupt timely and sufficient intake of nutrient during the immediate post-weaning period (Bolhuis et al., 2009). Numerous studies conducted for understanding prenatal learning process during gestation period as one of the way to improve food preferences (Smotherman, 1982; Robinson and Méndez-Gallardo, 2010).

Anise flavor or trans-anethole are selected in several studies to investigate perinatal learning mechanism for humans, dogs, and pigs due to their safety and strong odor (Schaal et al., 2000; Hepper and Wells, 2006; Oostindjer et al., 2009, 2010, 2011; Blavi et al., 2016). Humans and dogs have shown that prenatal exposure to anise flavor from the maternal diets may affect to an olfactory preference for anise flavor when they take foodstuff after birth (Schaal et al., 2000; Hepper and Wells, 2006). Similar results reported from pigs (Oostindjer et al., 2009, 2010, 2011). According to the study of Blavi et al. (2016), anethole was detected in amniotic fluid from sows fed treatment diet with flavored (containing >25% anethole and cinnamaldehyde and >10% eugenol) during late gestation period to farrowing. This study showed that amniotic fluid is pathway of certain flavors from maternal diet to fetuses in pigs. Because the composition exchange between fetuses and amniotic fluid occurred by fetal swallowing and micturition during the late gestation period (Robinson and Méndez-Gallardo, 2010). Also, few studies observed that prenatal exposure of anise flavors from maternal diets enhanced feed intake and growth performance of piglets (Langendijk et al., 2007; Oostindjer et al., 2009, 2010, 2011).

However, there are limited scientific results on the consecutive effect of dietary SA supplementation during gestation and proper supplementation range during post-weaning period for increase feed preferences and improve growth performance. The aim of this study was to investigate the proper supplementation

level of SA during post-weaning periods for imprinting impacts and to gain more insight in the mechanism of prenatal flavor learning.

2. MATERIALS AND METHODS

All experimental procedure performed in this study was approved by Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC). Feeding trial of sows and weaning pigs were conducted in a multisite production system. The sow experiment was conducted at the Jacob Swine Research Farm, located in Eumseong-gun, Chungcheongbuk-do, Korea Republic. The weaning pig experiment was conducted at the Dae-woo Swine Research Farm, located in Muan-gun, Jeollanam-do, Korea Republic.

The plant extract using in this study named Revest® Arom 1033 Anise P (BIOMIN Phytogenics GmbH, Stadtoldendorf, Germany), which involved 15.0% of natural flavor extracts from star anise (*Illicium verum*). The 85.0% of this product composed with binder and carrier which were silica dioxide and sodium chloride.

2.1 Animal

Experimental sows were introduced in an individual gestation stall in an environmentally controlled barn. Estrus was diagnosed twice daily in the presence of a mature boar, using the backfat pressure test. Sows were twice served artificial insemination (AI) with fresh diluted semen (Darby A.I. center, Chungju-si, Chungcheongbuk-do, Korea Republic) at 12 h intervals. Pregnancy of the sows was diagnosed by an ultrasound analyzer (Easyscan, Dong-jin BLS Co., Ltd., Gwangju-si, Gyeonggi-do, Korea Republic) on d 28 and 35 postcoitum.

On day 90 of gestation, the sows were allotted to one of two treatments, either 0% or 0.1% SA supplementation (Table 1) by the basis of body weight (BW), backfat thickness (BFT) and parity in a completely randomized design (CRD). As a result, a total of 50 multiparous gestating sows (Yorkshire x Landrace, avg. BW 256.3±23.6 kg) were used in this experiment. On day 110 of gestation, sows were

introduced into individual farrowing crates (2.2 m × 1.5 m). Also, daily feed intake was gradually reduced by 0.2 kg/day for each sow until the day of delivery.

Within 24 h post-farrowing, sows in each treatment group were supplemented by corn-soybean meal based lactation diets. The lactation diet was gradually increased from 1.0 kg/d by 1 kg/d until 5 d postpartum with a free access to water. For piglets, procedures including Fe-dextran (150 ppm) injection, ear notching, needle teeth clipping and tail docking were practiced after 3 days birth. Additionally, piglets were cross-fostered among treatments within 24 hrs after birth to balance suckling intensity across sows with equalization of litter size, and thus to minimize any impact of initial litter size potentially affecting litter growth.

After weaning, pigs were moved into another weaning pigs' house. A total of 120 weaning pigs ([Yorkshire×Landrace]×Duroc, avg. BW 7.78±0.762 kg) were used to investigate imprinting effect on weaning pigs. The weaning pigs were allotted to 4 treatments with 5 replicates (6 pigs per pen) in a randomized complete block design (RCBD) by sex and body weight.

2.2 Housing

The experimental sows were housed in a gestation barn with an individual crate (2.4 m × 0.64 m) with a fully slatted concrete floor. Room temperatures and ventilation rates were measured and determined with sensors, which were installed near the sows and were manipulated by an automatic climate control system (KO-850, KUN OK Co., Ltd., Nonsan-si, Chungcheongnam-do, Korea Republic). The average temperature during the entire experimental period was 20.0℃. Feed was accurately weighed by an electric-scale (SW-1W, CAS Co. Ltd., Yangju-si, Gyeonggi-do, Korea Republic), and feed was provided 2.4kg once a day (08:00) by feed buckets through an individual feeder with one waterer per sow.

At 110 day of gestation, all sows were moved to the farrowing crates (2.20 m×0.65 m) with partition walls (2.50 m×1.80 m) after washing and disinfecting their body. Lactating sows were provided the feed *ad libitum* after 5 days parturition and waterer were provided for sows and their progeny. During lactation, the room

temperature and air conditioning of the farrowing barn were kept automatically at 25 ± 3℃ and warming box for piglets were kept 30-32℃ by heating lamps and ventilation fans. After weaning, sows were moved to the breeding barn again for next AI service and weaning pigs were weaned from dam and housed in a plastic floor pen (1.54 m× 1.96 m), equipped with a feeder and a nipple drinker to allow freely access to feed and water during the four week experimental period. The ambient temperature in the weaning house was kept 31℃ during the first 7 days and lowered 1℃ every week to 28℃.

2.3 Experimental design and diets

The experimental treatment was designed to investigate whether anise extracts supplementation to sows during late-pregnancy induce imprinting effects on piglets. The factors were supplementation of SA either in late gestation (0% or 0.1%) and weaning period (0.02% or 0.04%), respectively. An overview of all experimental treatments and procedures is given in Figure 1.

The chemical composition and formula of experimental diets were shown in Table 1. SA was top-dressed on both gestation and weaning diet. Gestation diet contained 3,075 ME kcal/kg and 13.0% of crude protein (CP), 0.79% of lysine, 0.49% of methionine+cysteine, 0.57% of threonine, and 0.15% of tryptophan, respectively. Lactation diet contained 3,116 ME kcal/kg and 17.7% of CP, 1.02% of lysine, 0.63% of methionine+cysteine, 0.66% of threonine, and 0.22% of tryptophan, respectively. Gestation diets were provided daily at 2.4 kg/day. Lactation and weaning diet was provided *ad libitum* and water was available freely in both periods. Weaning pig's diets contained 3,410 ME kcal/kg and 20.04% of CP, 1.59% of lysine, 0.89% of methionine+cysteine, 0.96% of threonine, and 0.26% of tryptophan, respectively. SA 0.02% or 0.04% was top-dressed for each treatment. All other nutrients were met or exceeded requirements of NRC (2012).

2.4 Measurements and sample collection

In the gestation barn, BW and BFT of sows were measured at days 90, and 110 of gestation and 12 h, 21 d postpartum. BFT was measured at the P_2 position (last rib, 65 mm from the center line of the back) on both sides of the back bone using an electric measuring device (Lean-Meater®, Renco Corp., Minneapolis, MN, USA). Values from the two measurements were averaged to record a single BFT. Reproductive performance included the number of piglets born alive, stillborn, mummies, and losses. Within 24 h after birth, the litters were weighed individually by electric-scale (SW-1W, CAS Co. Ltd., Yangju-si, Gyeonggi-do, Korea Republic). Litter and piglet birth BW, 21d of lactation BW, and weight gain from birth-to-21d lactation were calculated. To observe piglet uniformity, coefficient of variation (CV), standard deviation (SD) was calculated at piglet birth and 21d lactation BW. Body weight and feed consumption of weaning pigs were recorded at d 0, 7, 14 and 28 post-weaning to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F ratio). Also, coefficient of variation (CV), standard deviation (SD) was calculated by body weight of weaning pigs at each phase (0, 1, 2, 4 weeks).

Sow (n=4 for each treatment) blood collection was taken by venipuncture of the jugular vein using 10 ml disposable syringes at the same time of measuring the BW and BFT. Suckling piglet (n=4 for each treatment) blood was collected from the anterior vena cava using 3 ml disposable syringes at 12 h, 21 d postpartum. Weaning pig (n=4 for each treatment) blood was collected from the anterior vena cava using 5 ml disposable syringes at initial, 1 week, 2 week, 4 week after 3 hours fasting. All samples were enclosed into serum tube (SST^{TM}Ⅱ Advance, BD Vacutainer, Becton Dickinson, Plymouth, UK) as well as ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer K_2E, Becton Dickinson, Plymouth, UK) and centrifuged at 3000 rpm and 4℃ for 10 min (5810R, Eppendorf, Hamburg, Germany) after clotting at room temperature for 30 min. The upper liquid (serum) of the blood was separated to a microtube (Axygen, Union City, CA, USA) and stored at −20℃ freezer until later

analysis.

Colostrum samples were taken from functional mammary glands at 24 hrs postpartum and milk samples were taken at 21 d postpartum. Colostrum and milk were collected from the first and second teats after an intravascular injection with 5 IU oxytocin (Komi oxytocin inj., Komipharm International Co., Ltd., Siheung-si, Gyeonggi-do, Korea Republic) in the ear. After collection, samples were stored in a freezer (−20℃) until further analysis. Proximate analysis of colostrum and milk was determined using a Milkoscan FT 120 (FOSS, Hillerod, Denmark).

2.5 Blood analysis

The serum superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in sow serum were measured using commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. Total antioxidant status (TAS) was measured using an automated method (Erel, 2004). The Rel Assay (Rel Assay®, Diagnostics kits, Mega Tıp, Gaziantep, Turkey) was used. This method is based on the reaction of ABTS (2.2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) with peroxidase and H_2O_2 to produce ABTS radical cation. Plasma for cortisol concentration was measured using a commercially available ELISA kit (swine cortisol ELISA kit, Endocrine Technologies, U.S.).

2.6 Statistical analysis

Sow data was analyzed as a completely randomized design with SA supplementation (0 or 0.1%) in gestation diet. Performances of SA supplementation in late gestation diet during gestation to lactation period were analyzed via pairwise T-test including physiological changes, reproductive performance, bloods and milk analyses. Individual sow was considered as the experimental unit.

Data of weaning pig including growth performance and blood analysis was analyzed as a randomized complete block design with two-way ANOVA. The pen of pigs was used as the experimental unit in growth performance, and individual piglet was used as the experimental unit in blood analysis. The significant difference was

set at $P < 0.05$, and tendency were determined if $0.05 \leq P < 0.10$. All the data was analyzed by the General Linear Model (GLM) procedure of SAS (version 9.4: SAS Institute Inc., Cary, NC, USA).

3. RESULTS

In late gestation, there were no significant differences in physiological response in relation to the effect of supplementing SA (Table 4). And this study did not find any significant difference in reproductive performance from supplementing SA (Table 5). In this report, we did not observe significant difference between treatments on backfat thickness change and body weight change during lactation period (Table 6). And there were no significant difference in number of piglets, litter weight change, and piglet weight during lactation period (Table 7).

The results of milk composition of lactating sows fed diets with or without supplemental SA were in Table 8. The casein and protein of colostrum and ordinary milk were not affected by dietary treatment. On the other hand, when sows were fed control diet, high level fat contents and total solid in sow milk at 21d lactation were observed (P=0.01, respectively). There were no significant change of solid not fat and FFA. However, sow milk density was higher in sows fed SA during late gestation (P=0.03).

The effects of SA supplementation during late gestation period on the growth performance of weaning pigs were summarized in Table 9. There were no significant differences among treatments in body weight change during 4 weeks after weaning. However, supplementing 0.04% SA during post-weaning tended to enhance ADG at two to four weeks (Weaning, P=0.09). Additionally, prenatal exposure during late gestation group showed lower ADFI at initial time to one week (Gestation, P=0.09). And supplementing 0.02% SA during post-weaning increased gain to feed ratio significantly at initial time to one week (Weaning, P=0.02).

The effects of SA supplementation during late gestation and different diet levels during post-weaning on piglet uniformity of weaning pigs were summarized in

Table 10. In these results showed that prenatal exposure of SA improve the coefficient of variation at weaning and 4 week after weaning, respectively (Gestation, $P=0.02$, $P<0.01$). And the concentration level of cortisol observed significant decrease by prenatal exposure and high level supplementation on piglet diets at 4 weeks after weaning (GxW, $P<0.01$, Table 11.).

4. DISCUSSION

This study investigated the effects of supplementing SA in the sow diet during late gestation period on physiological response and reproductive performance, as well as the subsequent growth and health in the post-weaning period of their progeny. Result from this study indicated that that supplementing SA to the gestation diet did not affect significant difference on reproductive performance. However, this study observed that composition of milk affected by SA supplementation during late gestation. Moreover, in post-weaning period, pigs with prenatal anise exposure decreased stress related hormones at 4 week after weaning, resulting enhanced uniformity of piglets.

There were very few studies about SA supplementation on sow performance have been conducted. According to the Wang et al. (2015), there were no significant differences from two diets groups supplemented with 0.5 % SA or basal diet during late gestation period. However, Wang et al. (2015) observed that supplementing SA during lactation period improved milk yield, average daily feed intake of lactation sows and improved weaning weight of piglets. Similar results were also reported by Lei et al. (2015) that SA supplementation increased ADFI of lactation sows and decreased backfat loss of lactation sow during lactation period. Supplementation of SA showed the similar effects like as phytoestrogen substances (Wang et al., 2015). Estrogen and phytoestrogen controls expressions of the IGF system in vitro and in vivo and improve pre- and postnatal growth in newborn male piglets (Liu et al., 1999; Ren et al., 2001). In this study, we did not observe any significant differences of backfat losses and body weight change in sows during

gestation and lactation.

The data from current experiment showed that SA supplementation during late gestation decreased fat and total solid of milk composition at 21 day of lactation, respectively (P=0.01). On the other hand, density of milk increased significantly (P=0.03). Similar result reported by Matysiak et al. (2012) noted that plant extract mainly consist of carvacrol, cinnamaldehyde and capsicum oleoresin in the lactation diet of sow increased the concentration of lactose in the milk. Additionally, Ariza-Nieto et al. (2011) indicated that reduction of fat contents in sow milk on d 7 and d 14 in lactation sows supplementing diet with oregano essential oil during lactation period. Fat concentration in sow milk increased with the passage of lactation time in normal condition (Hurley, 2015). According to the review of Hurley (2015), fat contents is the most variable components in sow milk and affected by several factors; stage of lactation, gestation diets, lactation diets, supplementation of folic acid, breeding, milk yield, and environmental temperature. There were no clear evidences for explain that reduction of fat level in milk (Bauman and Griinari, 2001). Further research is needed to investigate the relationship between plant extract and reduction fat contents in sow milk.

In the current experiment, piglet supplementing diets with 0.04% SA tended to increase ADG during two to four week after weaning (Weaning, P=0.09). Al-Kassie (2008) reported similar results in broilers, broilers supplementing with 0.1% SA showed significant improvement of ADG. However, we observed low gain to feed ratio in 0.04% SA supplementation group significantly (Weaning, P=0.02). Based on the result of these studies, SA may have beneficial effects as a growth promoter, but inclusion rate should be considered for negative impacts.

Also, in this study indicated that supplementation SA during late gestation improved uniformity of their progenies at weaning and 4 week after weaning significantly (Gestation, P<0.01). Improvement of uniformity is a priority goal in swine production, because high variation of pigs weight increase management cost in farm from less efficient of dietary nutrient, barn utilization, and labor (O'Quinn et al., 2001). There was no previous study that SA supplementation during late gestation

and re-exposure of flavor with SA supplementation influenced piglet uniformity. One of possible hypothesis is that stress reduction of piglet through re-exposure of anise flavor improves the uniformity. Oostindjer et al. (2011) found stress relationship with prenatal exposure of anise flavor and re-exposure after weaning. According to the experimental results of this research, flavor-exposed piglets tended to show a faster decrease in salivary cortisol levels after weaning. Initial stress response was similar for all group, however treatment group with re-exposure of familiar flavor decreased salivary cortisol quickly. We did not observe consistent result from cortisol level from weaning piglet, however, piglets exposed SA through maternal diet and supplemented with 0.04% SA showed lowest cortisol level at 4 week after weaning (GxW, $P<0.01$). Kelley (1980) reported that diverse types stressors from environmental challenges (fear, frustration, maintenance of social dominance of hierarchies, separation from their mother, and etc.) influenced specific immune responses in the body. These previous studies are the clues to understanding relationship among prenatal learning, stress level, and uniformity of young pigs. However, additional research is needed to investigate clear understanding about uniformity and stress reduction.

5. CONCLUSION

Supplementation of SA in the sow diet during late gestation influenced litter weight, milk fat composition at 21d lactation sow, and piglet performances. For piglets, SA reduced weaning stress as well as enhanced uniformity when piglets consumed diet with SA. There were no significant differences from SA inclusion level on piglet diets. Therefore, less than 0.02% SA supplementation in post-weaning diets recommended to observe imprinting impacts in weaning pigs. However, further studies are needed for evaluation of relationship between stress and uniformity.

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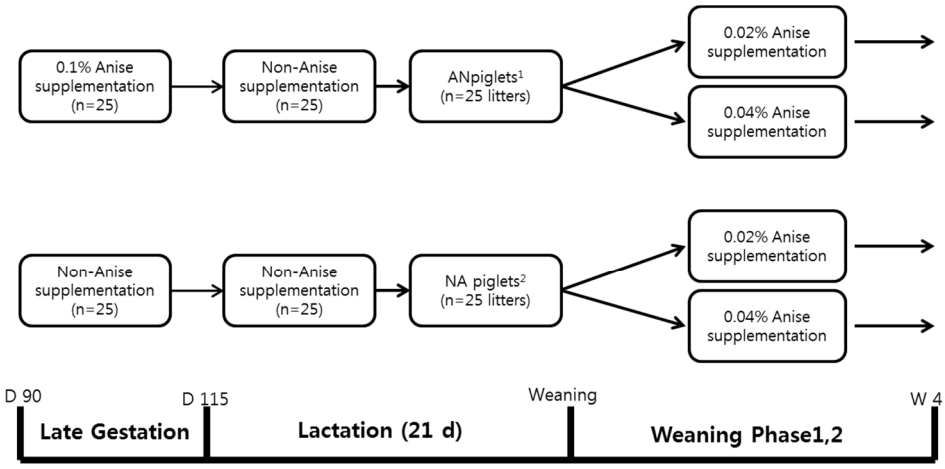

[1] piglets from gestation basal diet +0.1%SA, lactation basal diet
[2] piglets from gestation basal diet, lactation basal diet

Figure 1. The experimental design and the timeline of feeding regimen.

Table 1. The formulas and chemical composition of gestation and lactation diet[1]

Item	Gestation		Lactation
	Control	Anise	
Ingredient, %			
Corn, yellow	72.55	72.55	63.7
Soybean meal, 45% CP	14.75	14.75	27.2
Wheat mill run	6.0	6.0	2.8
Animal fat	2.6	2.6	2.8
Monodicalcium phosphate	1.8	1.8	1.5
Limestone	1.3	1.3	1.3
Lysine sulfate, 51%	0.29	0.29	0.1
Salt	0.3	0.3	0.3
Choline chloride, 50%	0.1	0.1	0.1
L-threonine, 98%	0.1	0.1	0
Anise Extract	0	0.1	0
Vit. Mix.[2]	0.06	0.06	0.05
Min. Mix.[3]	0.15	0.15	0.15
Total	100.0	100.1	100.0
Chemical composition[4]			
ME, kcal/kg	3075.00	3075.00	3116.40
Crude protein, %	13.00	13.00	17.70
Calcium, %	0.85	0.85	0.83
Phosphorus, %	0.71	0.71	0.67
Lysine, %	0.79	0.79	1.02
Methionine+cysteine, %	0.49	0.49	0.63
Threonine, %	0.57	0.57	0.66
Tryptophan, %	0.15	0.15	0.22

[1]Treatments: Gestation diets were fed 2.4kg/day in two separate meals; lactation diets were fed *ad libitum* up to weaning at 21days. Star anise diets were supplemented with 0.1% anise extract.

[2]Provided per kg of diet:

Gestation: vitamin A, 10,800 IU; vitamin D_3, 2,400 IU; vitamin E, 72 IU; vitamin K, 3.6 mg; vitamin B_2, 7.2 mg; vitamin B_6, 4.8 mg; vitamin B_{12}, 30 µg; pantothenic acid, 24 mg; biotin, 324 µg; niacin, 48 mg; folic acid 3.12 mg; thiamine, 1.56mg.

Lactation: vitamin A, 9,000 IU; vitamin D_3, 2,000 IU; vitamin E, 60 IU; vitamin K, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 25 µg; pantothenic acid, 20 mg; biotin, 270 µg; niacin, 40 mg; folic acid 2.6 mg; thiamine, 1.3 mg.

[3]Provided per kg of diet: Fe, 165 mg; Mn, 60 mg; Zn, 99 mg; Cu, 7.5 mg; Se, 450 µg; I, 1 mg.

[4]Calculated values.

Table 2. The formulas and chemical composition of weaning phase 1 diet[1]

Item	Treatment		
	Control	Star anise 0.02%	Star anise 0.04%
Ingredient, %			
Corn, yellow	47.363	47.363	47.363
Soybean meal, 45% CP	15	15	15
Permeate SBM, 54%	5.5	5.5	5.5
Whey permeate	7.5	7.5	7.5
Lactose	5	5	5
Whey	2	2	2
Wheat mill run	3	3	3
Plasma protein	4.5	4.5	4.5
White fishmeal	2.5	2.5	2.5
Coconut fat	2	2	2
Vegetable oil	1.5	1.5	1.5
Monodicalcium phosphate	0.72	0.72	0.72
Limestone	0.5	0.5	0.5
Lysine sulfate, 51%	0.783	0.783	0.783
Salt	0.3	0.3	0.3
Choline chloride, 50%	0.14	0.14	0.14
L-threonine, 98%	0.159	0.159	0.159
Anise Extract		0.02	0.04
Organic acid	0.4	0.4	0.4
Enzyme mix	0.14	0.14	0.14
Probiotic	0.154	0.154	0.154
Methionine hydroxy analogue, 98%	0.219	0.219	0.219
Zinc oxide, 90%	0.313	0.313	0.313
Yucca powder	0.013	0.013	0.013
Tryptophan, 10%	0.096	0.096	0.096
Vit. Mix.[2]	0.1	0.1	0.1
Min. Mix.[3]	0.1	0.1	0.1
Total	100.0	100.02	100.04
Chemical composition[4]			
ME, kcal/kg	3410	3410	3410
Crude protein, %	20.04	20.04	20.04
Calcium, %	0.62	0.62	0.62
Phosphorus, %	0.63	0.63	0.63
Lysine, %	1.59	1.59	1.59
Methionine+cysteine, %	0.89	0.89	0.89
Threonine, %	0.96	0.96	0.96
Tryptophan, %	0.26	0.26	0.26

[1]Treatments: The first factor was SA supplementation (0% or 0.1%) in late-gestation period of sows and the second factor was SA supplementation (0.02% or 0.04%) in post-weaning period.

[2]Provided per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 60 IU; vitamin K, 3.5 mg; vitamin B_2, 8 mg; vitamin B_6, 2 mg; vitamin B_{12}, 35 µg; pantothenic acid, 25 mg; biotin, 100 µg; niacin, 50 mg; folic acid 1.1 mg; thiamine, 1.5mg.

[3]Provided per kg of diet: Fe, 100 mg; Mn, 50 mg; Zn, 50 mg; Cu, 80 mg; Se, 400 µg; I, 1 mg.

[4]Calculated values.

Table 3. The formulas and chemical composition of weaning phase 2 diet[1]

Item	Treatments		
	Control	Star anise 0.02%	Star anise 0.04%
Ingredient, %			
Corn, yellow	42.342	42.342	42.342
Soybean meal, 45% CP	18.47	18.47	18.47
Wheat flour	10	10	10
Permeate SBM, 54%	5	5	5
Whey permeate	5.36	5.36	5.36
Lactose	2	2	2
Whey	2	2	2
Wheat mill run	1.5	1.5	1.5
Rice protein concentrate	1	1	1
White fishmeal	2	2	2
Coconut fat	1.2	1.2	1.2
Vegetable oil	1	1	1
Animal fat	1.8	1.8	1.8
Sugarcane molasses	2.5	2.5	2.5
Monodicalcium phosphate	0.66	0.66	0.66
Limestone	0.5	0.5	0.5
Lysine sulfate, 51%	0.608	0.608	0.608
Salt	0.26	0.26	0.26
Choline chloride, 50%	0.131	0.131	0.131
L-threonine, 98%	0.169	0.169	0.169
Anise Extract		0.02	0.04
Organic acid	0.4	0.4	0.4
Enzyme mix	0.194	0.194	0.194
Probiotic	0.154	0.154	0.154
Methionine hydroxyl analogue,84%	0.171	0.171	0.171
Zinc oxide, 90%	0.313	0.313	0.313
Tryptophan, 10%	0.035	0.035	0.035
Yucca powder	0.013	0.013	0.013
Vit. Mix.[2]	0.12	0.12	0.12
Min. Mix.[3]	0.1	0.1	0.1
Total	100	100.02	100.04
Chemical composition[4]			
ME, kcal/kg	3396.3	3396.3	3396.3
Crude protein, %	19.24	19.24	19.24
Calcium, %	0.59	0.59	0.59
Phosphorus, %	0.57	0.57	0.57
Lysine, %	1.31	1.31	1.31
Methionine+cysteine, %	0.79	0.79	0.79
Threonine, %	0.85	0.85	0.85
Tryptophan, %	0.23	0.23	0.23

[1]Treatments: The first factor was SA supplementation (0% or 0.1%) in late-gestation period of sows and the second factor SA supplementation (0.02% or 0.04%) in post-weaning period.

[2]Provided per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 2,400 IU; vitamin E, 72 IU; vitamin K, 4.2 mg; vitamin B_2, 9.6 mg; vitamin B_6, 2.4 mg; vitamin B_{12}, 42 µg; pantothenic acid, 30 mg; biotin, 120 µg; niacin, 60 mg; folic acid 1.3 mg; thiamine, 1.8mg.

[3]Provided per kg of diet: Fe, 100 mg; Mn, 50 mg; Zn, 50 mg; Cu, 80 mg; Se, 400 µg; I, 1 mg.

[4]Calculated values.

Table 4. Effect of star anise supplementation on changes of sow body weight and backfat thickness during gestation

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=25)	Anise (*n*=25)		
Body weight, kg				
90d gestation	255.6	256.2	3.38	0.93
110d gestation	263.4	257.2	5.97	0.60
BW gain(90-110d)	7.8	1.0	5.28	0.52
Backfat thickness, mm				
90d gestation	22.4	21.8	0.81	0.70
110d gestation	22.9	22.7	0.76	0.91
BF gain(90-110d)	0.5	0.9	0.30	0.46

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.

Table 5. Effect of star anise supplementation on reproductive performance and uniformity of progeny

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=25)	Anise (*n*=25)		
No. of piglets / litter				
Total born	14.16	13.88	0.539	0.79
Stillborn	1.04	0.76	0.165	0.40
Mummy	0.40	0.16	0.103	0.25
Born alive	12.72	12.96	0.459	0.79
Total litter weight, kg	19.32	19.85	0.517	0.61
Alive litter weight, kg	18.06	18.93	0.494	0.38
Uniformity				
SD[3], g	331.1	341.1	16.51	0.76
CV[4], %	23.21	23.48	1.17	0.91

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.
[3] Standard deviation.
[4] Coefficient of variation.

Table 6. Effect of star anise supplementation on changes of sow body weight, backfat thickness and feed intake during lactation

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=25)	Anise (*n*=25)		
Body weight, kg				
24hrs postpartum	240.9	245.6	3.55	0.51
21d lactation	228.9	237.4	3.94	0.28
BW changes (0-21d)	-12.0	-8.2	2.34	0.42
Backfat thickness, mm				
24hrs postpartum	22.6	22.1	0.96	0.81
21d lactation	19.6	20.1	0.90	0.76
BF changes (0-21d)	-3.0	-2.0	0.44	0.26
ADFI, kg/d	5.08	5.24	0.162	0.61

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.

Table 7. Effect of star anise supplementation on litter performance during lactation period

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=25)	Anise (*n*=25)		
No. of piglets				
Initial (after-fostering)	11.33	11.83	0.173	0.15
Final (21d lactation)	10.54	11.17	0.218	0.15
Litter weight, kg				
24hrs postpartum	16.57	17.63	0.413	0.20
21d lactation	62.25	66.50	1.742	0.22
Weight gain (0-21d)	45.68	48.87	1.582	0.31
Piglet weight, kg				
24hrs postpartum	1.47	1.50	0.036	0.65
21d lactation	5.91	5.99	0.131	0.75
Weight gain(0-21d)	4.44	4.49	0.116	0.82

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.

Table 8. Effect of star anise supplementation on milk composition during lactation

Item	Treatment[1]		SEM[2]	P-value
	Control (*n*=25)	Anise (*n*=25)		
Casein, %				
Colostrum	7.80	7.37	0.879	0.82
Milk at 21d lactation	4.69	4.61	0.074	0.62
Fat, %				
Colostrum	6.62	6.52	0.404	0.91
Milk at 21d lactation	8.26	6.09	0.513	0.01
Protein, %				
Colostrum	10.45	9.68	1.251	0.78
Milk at 21d lactation	4.92	4.89	0.145	0.93
Lactose, %				
Colostrum	3.51	3.91	0.279	0.50
Milk at 21d lactation	5.72	6.10	0.120	0.11
Total solid, %				
Colostrum	23.50	22.79	1.412	0.82
Milk at 21d lactation	19.92	18.09	0.422	0.01
Solid not fat, %				
Colostrum	14.35	13.97	1.049	0.87
Milk at 21d lactation	10.69	11.13	0.149	0.15
Density				
Colostrum	1038	1039	2.2	0.87
Milk at 21d lactation	1030	1036	1.5	0.03
FFA[3], %				
Colostrum	3.28	3.69	0.262	0.47
Milk at 21d lactation	7.60	7.92	0.614	0.81

[1] Control = corn-SBM based diet; Anise = basal diet with 0.1% SA.
[2] Standard error of means.
[3] Free fatty acid.

Table 9. Effect of star anise supplementation in sow diet and supplementation levels weaning diet on growth performance of weaning pigs

Gestation	Control		Anise			P-value[2]		
Weaning	**0.02%**	**0.04%**	**0.02%**	**0.04%**	**SEM[1]**	**G**	**W**	**GxW**
Body weight, kg								
Initial	7.75	7.75	7.77	7.77	0.159	0.93	0.99	0.99
1 week	8.98	8.93	8.93	8.72	0.158	0.71	0.67	0.82
2 week	12.08	12.07	11.90	11.74	0.213	0.59	0.85	0.86
4 week	18.12	18.98	18.17	18.55	0.381	0.81	0.45	0.77
Average daily gain, g								
0-1 week	171	168	165	135	7.4	0.21	0.27	0.36
1-2 week	442	449	423	446	14.2	0.73	0.63	0.80
2-4 week	432	493	447	487	14.3	0.88	0.09	0.70
0-4 week	370	401	371	385	9.5	0.70	0.28	0.67
ADFI, g								
0-1 week	206	223	190	196	6.1	0.09	0.33	0.67
1-2 week	583	584	542	543	16.1	0.23	0.96	0.99
2-4 week	745	790	753	820	23.4	0.70	0.27	0.82
0-4 week	570	597	559	595	14.8	0.84	0.33	0.89
Gain : feed ratio								
0-1 week	0.832	0.754	0.874	0.686	0.0293	0.81	0.02	0.31
1-2 week	0.760	0.768	0.778	0.822	0.0134	0.19	0.35	0.50
2-4 week	0.580	0.639	0.598	0.595	0.0177	0.74	0.46	0.42
0-4 week	0.650	0.679	0.665	0.649	0.0128	0.79	0.81	0.41

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, W: SA 0.02% supplementation or 0.04% during post-weaning, G x W: interaction between SA supplementation during gestation and post-weaning.

Table 10. Effect of star anise supplementation in sow diet and supplementation levels weaning diet on weaning pigs' uniformity

Gestation	Control		Anise			P-value[2]		
Weaning	**0.02%**	**0.04%**	**0.02%**	**0.04%**	**SEM[1]**	**G**	**W**	**G x W**
SD[3], kg								
Initial	0.310	0.373	0.265	0.201	0.0279	0.05	0.98	0.24
1 week	0.494	0.598	0.628	0.540	0.0270	0.48	0.88	0.08
2 week	1.140	1.146	1.008	0.920	0.0605	0.16	0.74	0.70
4 week	2.626	2.370	1.688	1.390	0.1658	<0.01	0.30	0.93
CV[4], %								
Initial	4.037	4.832	3.366	2.519	0.3449	0.02	0.96	0.20
1 week	5.468	6.706	7.044	6.166	0.2962	0.37	0.75	0.08
2 week	9.454	9.522	8.412	7.876	0.4864	0.19	0.81	0.76
4 week	14.814	12.680	9.512	7.486	1.0263	<0.01	0.24	0.97

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, W: SA 0.02% supplementation or 0.04% during post-weaning, G x W: interaction between SA supplementation during gestation and post-weaning.
[3] Standard deviation.
[4] Coefficient of variation.

Table 11. Effect of star anise supplementation in gestation diet and supplementation levels weaning diet on blood cortisol in weaning pigs

Gestation	Control		Anise			P-value[2]		
Weaning	0.02%	0.04%	0.02%	0.04%	SEM[1]	G	W	G x W
Cortisol, μg/dL								
Initial	7.59	7.59	7.59	7.59				
1 week	2.75	2.50	3.20	1.93	0.222	0.89	0.09	0.25
2 week	3.85	3.76	3.35	2.93	0.384	0.42	0.76	0.84
4 week	2.48	3.34	4.40	1.38	0.355	0.97	0.06	<0.01

[1] Standard error of means.
[2] G: SA 0.1% supplementation or not during gestation, W: SA 0.02% supplementation or 0.04% during post-weaning , G x W: interaction between SA supplementation during gestation and post-weaning.

Chapter V.Effects of Star Anise (*Illicium verum*) Supplementation during Growing to Finishing Periods after Prenatal Exposure of Star Anise on Growth Performance and Meat Quality in Pigs

ABSTRACTS: The effects of prenatal and postnatal exposure with star anise (SA) flavor for young animals have been reported. However, the information on long term effects of re-exposure of flavor after prenatal exposure and meat quality impacts by long term supplementation of SA flavor is lacking. To investigate long term effect of SA in meat quality, a total of 120 growing pigs ([Yorkshire × Landrace] × Duroc), averaging 24.83±2.95 kg body weight were used in feeding trial. The experimental design was composed by two factors with factorial design for evaluating imprinting effects on growing-finishing pigs. The first factor was SA supplementation (0% or 0.1%) in late-gestation period of sows and the second factor was SA supplementation (0% or 0.02%) in growing-finishing period. There were no significant difference in body weight (BW) and average daily feed intake (ADFI) in growing to finishing phase (0-13week). However, ADG was increased as prenatal exposure and supplementation level of SA during growing-finishing period in 11-13week (GxL, P=0.03) and 8-13 week (GxL, P=0.04). In addition, there was an increase gain to feed ratio (G:F ratio) as prenatal exposure in 8-13week (Gestation, P=0.03) and overall period (Gestation, P=0.02). Prenatal exposure and re-exposure of SA flavor group showed improvement 46.67% of uniformity than control group (Gestation, P=0.05). Plasma cortisol was higher at prenatal SA flavor than control diets (Gestation, P=0.02) at 3 week. However, control diet treatment showed higher cortisol level at 12 week (Gestation, P=0.02). Prenatal SA flavor treatment showed higher superoxide dismutase (SOD) than control diet treatment at 9 week (Gestation, P<0.01). The lower pH of pork on 24 hour postmortem of carcass as prenatal exposure of SA through maternal diets observed (Gestation, P=0.03). Crude protein of pork decreased (Gestation, P=0.03) as prenatal impacts and crude fat increased as prenatal impacts

(Gestation, P=0.03). The result suggested that supplementation SA during late gestation and re-exposure of SA flavor or supplementation during growing-finishing pigs improved growth performance of pigs without negative impacts of pork quality.

Key words: Growing-finishing pigs, Imprinting, Pork quality, Star anise, Stress, Uniformity

1. INTRODUCTION

The use of phytogenic additives in animal diet is major trend since antibiotics are banned. Phytogenic additives contain a wide range of bioactive compounds which can improve animal performance by multiple actions (Steiner et al., 2009). Several researches demonstrated phytogenic additives support the animal health through antioxidative action (Padmashree et al., 2007; Windisch et al., 2008), antimicrobial action (Hammer et al.1999), growth promoting (Namkung et al.,2004; Lien et al., 2007), and imprinting impacts (Langendijk et al., 2007; Oostindjer et al., 2010).

Environment stress factors comes from heat, cold, mixing, weaning, noise and feeding change stress (Kelley, 1980). These stress effects are responsible that the growing-finishing pig is often in negative feed intake and poor growth performance (Hyun et al., 1998).

In a previous study, several researches demonstrated that prenatal exposure of flavor through maternal diet could improve feed acceptance and improve adaptation to re-exposure of flavor to their progeny (Langendijk et al., 2007; Oostindjer et al., 2009). In addition, prenatal exposure of anise flavor through the maternal diet increased feed intake and body weight and reduced stress-induced behaviors in newly weaned piglet (Oostindjer et al., 2010, 2011). However, the information on the long term effects of re-exposure of star anise (SA) flavor after prenatal exposure and meat quality impacts is lacking.

The objectives of this study were to determine 1) the effects of SA supplementation during growing to finishing periods after prenatal exposure of SA during late gestation on growth performance of growing-finishing pigs, and 2) the effects of meat quality from SA supplementation during growing to finishing periods.

2. MATERIALS AND METHODS

All experimental procedure performed in this study was approved by Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC). Feeding trial of sows and growing-finishing pigs were conducted in a multisite production system. The sow experiment was conducted at the Jacob Swine Research Farm, located in Eumseong-gun, Chungcheongbuk-do, Korea Republic. The growing-finishing pig experiment was conducted at the Dae-woo Swine Research Farm, located in Muan-gun, Jeollanam-do, Korea Republic.

2.1 Animals and star anise

A total of 120 growing pigs[(Yorkshire × Landrace) × Duroc] with average 24.83 ± 2.95 kg initial body weight used in a 13 week feeding trial, at a research farm located in Muan-gun, Jeollanamdo, South Korea. Growing pigs were allotted to one of four treatments in 5 replicates with 6 pigs per pen in a randomized complete block (RCB) design by body weight and sex.

The plant extract using in this study named Revest® Arom 1033 Anise P (BIOMIN Phytogenics GmbH, Stadtoldendorf, Germany), which involved 15.0% of natural flavor extracts from star anise (*Illicium verum*). The 85.0% of this product composed with binder and carrier which were silica dioxide and sodium chloride.

2.2 Experimental design and diet

The experimental design was composed by two factors with factorial design for evaluating imprinting effects on growing-finishing pigs. The first factor was SA supplementation (0% or 0.1%) in late-gestation period of sows and the second factor was SA supplementation (0% or 0.02%) in growing-finishing period. Growing-finishing period was composed with 2 phases such as growing phase (0-7 week), finishing phase (8-13 week).

The chemical composition and formula of experimental diets were shown in Table 1 and 2. SA 0.1% was top-dressed on gestation diet and 0.02% was top-dressed

on growing-finishing diet. Gestation diet contained 3,075 ME kcal/kg and 13.0% of crude protein (CP), 0.79% of lysine, 0.49% of methionine+cysteine, 0.57% of threonine, and 0.15% of tryptophan, respectively. Lactation diet contained 3,116 ME kcal/kg and 17.7% of CP, 1.02% of lysine, 0.63% of methionine+cysteine, 0.66% of threonine, and 0.22% of tryptophan, respectively. Gestation diets were provided daily at 2.4 kg/day in two equal meal. Lactation and growing-finishing diet was provided *ad libitum* and water was available freely in both period. Growing pig's diets contained 3,269 ME kcal/kg and 16.99% of CP, 1.09% of lysine, 0.66% of methionine+cysteine, 0.71% of threonine, and 0.19% of tryptophan, respectively. Finishing pig's diets contained 3,257 ME kcal/kg and 15.00% of CP, 0.93% of lysine, 0.58% of methionine+cysteine, 0.62% of threonine, and 0.16% of tryptophan, respectively. All other nutrients were met or exceeded requirements of NRC (2012).

2.3 Measurements and sample collection

Experimental period was consisted with 2 phases such as growing phase (0-7 week), finishing phase (8-13 week). For growing phase (0-7 week), growing pigs fed growing diet and finishing pigs fed finishing diet during finishing phase (8-13). Body weight and feed intake measured at 0, 3, 7, 10, 13 weeks considering phase and diet change time. In addition, average daily gain (ADG), average daily feed intake (ADFI), gain:feed ratio (G:F ratio) were measured at 0, 3, 7, 10, 13 weeks. Uniformity was calculated at initial date of experiment, 3, 7, 10, 13 week by measuring body weight at each time.

In each treatment, 4 pigs selected randomly for blood sampling and analysis of stress indicator and antioxidant indicator in plasma. Blood samples were taken at 0, 3, 7, 10, 13 weeks from anterior vena cava using 10 ml disposable syringes after 3 hours fasting and collected in serum tube (SSTTM Ⅱ Advance, BD Vacutainer®, UK). After then, samples were quickly centrifuged (Eppendorf centrifuge 5810R, Germany) for 15 minutes by 3,000 rpm at 4℃. After centrifuging, sera samples were transfer to 1.5ml microtube (Axygen®, CA, USA) and stored at -20℃ until further

analysis. The cortisol measured by automated analyzer for clinical chemistry (γ-counter, COBRA 5010 QUANTUM model). For analyzing superoxide dismutase (SOD), colorimetry analysis method (Microplate reader, VERSA Max, Molecular device, USA) were used with SOD assay kit (Cayman, USA).

In each treatment, 4 pigs were selected and slaughtered for the carcass analysis at the end of experiment. Longissimus muscle were used for pork quality measurements. The pork color measured by CIE color L, a and b values using a chromameter (Minolta CM-508i, Japan) at 3, 6, 12, 24 hours after slaughter. The pH was measured using a pH meter (Model 720, Thermo Orion, USA) at 0, 3, 6, 12, 24 hours after slaughter. The pork water holding capacity (WHC) was measured by centrifuge method. To evaluate water holding capacity, pork sample inputted into filter tube after grinding and heated 20 min at 80℃ on water bath, centrifuged 10 minute with 2,000 rpm at 10℃ using centrifuge (Eppendorf centrifuge 5810R, Germany). For calculating the cooking loss, pork were packed with polyethylene bag and heated in water bath until core temperature reached 72℃. Sample weight differences were recorded for cooking loss analysis. After the heat treatment, muscle fiber and the cores were sampled with 0.5 inch in diameter tube for shear force analysis (Warner Bratzler Shear, USA).

To evaluate fatty acids composition in pork loin, lipids in the loin samples (5 g) were extracted using 100 mL chloroform/methanol (2:1, v/v) according to the procedure used by Folch et al. (1957). After adding 0.88% NaCl, lipid samples were thoroughly mixed. After phase separation, the upper layer was removed and the remaining organic layer was dried under nitrogen flow. Extracted lipids were mixed with 2 mL of BF3-methanol (14%, w/w) then heated in a water bath at 85℃for 10 min. After cooling, 2 mL hexane and 5 mL DW were added to the samples and centrifuged (Hanil Co. Ltd.) at 2,149 g for 10 min. Then, the top layer of hexane containing fatty acid methyl esters was transferred to vials and separated using a gas chromatograph (HP 7890, Agilent Technologies, USA). A split inlet (split ratio, 50:1) was used to inject the samples into a capillary column (SPTM 2560 capillary column,

Supelco, USA) at film thickness of 100 m × 0.25 mm × 0.20 μm and at ramped oven temperature (100℃ for 5 min, increased to 240℃ at 4℃/min, and maintained for 20 min). Inlet temperature was 225℃. N2 was served as the carrier gas at a constant flow rate of 20 mL/min.

Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS). Each pork loin (5 g) was homogenized with 15 mL DW and 50 μL butylated hydroxyl toluene in ethanol. The homogenates (2 mL) were transferred to 15 mL test tubes and mixed with 4 mL 2-thiobarbituric acid (TBA) and trichloroacetic acid (20 mM TBA in 15% trichloroacetic acid). The tubes were heated in a water bath at 90℃ for 30 min, cooled, and centrifuged (HM-150IV, Hanil Co. Ltd., Korea) at 2,149 g for 10 min. The absorbance of the supernatants was measured at 532 nm using a spectrophotometer (Xma 3100). TBARS values were expressed as mg malondialdehyde/kg sample.

For sensory evaluation, loin samples were cut into pieces of similar size (1 cm × 3 cm × 0.5 cm) in a raw state, and then cooked to an internal temperature of 75ºC using a pan. Ten panelists, having at least 1 year of experience in analyzing meat quality in sensory evaluations, evaluated the samples. Sensory parameters, including color, flavor, taste, tenderness, and overall acceptability, were evaluated using a 9-point Hedonic scale, where 9 indicates “extremely like” and 1 indicates “extremely dislike.” Off-odor was assessed as follows: 9, very strong; and 1, no off-odor.

2.4 Statistical analysis

Data of growing-finishing pigs including growth performance and blood analysis was analyzed as a randomized complete block design with two-way ANOVA. The pen of pigs was used as the experimental unit in growth performance, and individual pig was used as the experimental unit in blood analysis. The significant difference was set at $P < 0.05$, and tendency were determined if $0.05 \leq P < 0.10$. All the data was analyzed by the General Linear Model (GLM) procedure of SAS

(version 9.4: SAS Institute Inc., Cary, NC, USA).

3. RESULTS

The effect of SA supplementation and imprinting impacts on growth performance was presented in Table 3. There were no significant differences in body weight (BW) and average daily feed intake (ADFI) in growing to finishing phase (0-13week). However, ADG was increased as prenatal exposure and supplementation level of SA during growing-finishing period in 11-13week (GxL, P=0.03) and 8-13 week (GxL, P=0.04). In addition, there was a linear increase gain to feed ratio (G:F ratio) as prenatal exposure in 8-13week (Gestation, P=0.03) and overall period (Gestation, P=0.02).

Prenatal exposure of SA flavor during late gestation period improved uniformity of growing-finishing pigs in overall period (Table 4). Prenatal exposure and re-exposure of SA flavor group showed improvement 46.67% of uniformity than control group at 13week (Gestation, P=0.05).

The effect of SA supplementation and imprinting impacts on stress and antioxidant parameter were shown in Table 5. Plasma cortisol was higher at prenatal SA flavor than control diets (Gestation, P=0.02) at 3 week. However, control diet treatment showed higher cortisol level at 12 week (Gestation, P=0.02). Prenatal SA flavor treatment showed higher superoxide dismutase (SOD) than control diet treatment at 9 week (Gestation, P<0.01).

The lower pH of pork on 24 hour postmortem of carcass as prenatal exposure of SA flavor through maternal diets observed (Gestation, P=0.03, Table 6).

The results of SA supplementation and imprinting impacts on pork color after slaughter were in Table 7. Prenatal exposure of SA and supplementation during growing-finishing period increased L value on 0 hour after slaughter (GxL, P=0.05). In addition, supplementation of SA during growing-finishing period increased a value on 0 hour after slaughter (G-F, P=0.03). Prenatal exposure of SA and supplementation level during growing-finishing period increased b value on 24 hour after slaughter

(GxL, P<0.01).

The effects of SA supplementation and imprinting impacts on pork composition and physiochemical property after slaughter are shown in Table 8. There were no significant difference in moisture and crude ash on pork. However, crude protein of pork decreased (Gestation, P=0.03) as prenatal impacts and crude fat increased as prenatal impacts (Gestation, P=0.03). There were no significant differences from physiochemical property.

The effect of SA supplementation and imprinting impacts on pork fatty acid composition was shown in Table 9. As a result of analysis of pork fatty acid, we observed interaction impacts of prenatal exposure and supplementation level at C20:0 (GxL, P=0.01). Moreover, C20:1 level increased as supplementation of SA flavor during late gestation (Gestation, P<0.01), supplementation of SA flavor during growing-finishing phase (G-F, P<0.01), respectively. And we observed interaction impacts of prenatal exposure and supplementation level on C20:1 (GxL, P<0.01).

There were no significant difference in TBARS after slaughter (Table 10) and sensory evaluation by trained panels (Table 11).

4. DISCUSSION

This is first report demonstrated effects of prenatal exposure and supplementation of SA on growth performance during growing-finishing period pigs. Val-Laillet et al. (2016) observed that piglet familiarized feed flavor from weaning had showed increasing response in the prefrontal, insular, and parahippocampal cotices, when they exposed to same flavor diets. This result show that flavor exposure influence to memory region in the brain. This observation provided a clue that imprinting effects can occur for a long time. The findings from this study support this hypothesis. The results indicate that prenatal exposure and supplementation of SA increase gain to feed ratio during overall period. Hepper and Wells (2006) observed opposite result that prenatal exposure of pups and re-exposure of same flavor did not influence feed preference 10 weeks after the last exposure. Therefore, memory

retention time of flavor may be different by species.

Also, this report showed that prenatal exposure and 0% consuming group has highest gain to feed ratio during overall time even though this group did not consume anise flavor again. Because it was difficult to block the odor in the experiment place by each treatment, non-prenatal exposure group showed similar results with re-exposure group. Similar result reported that re-exposure anise flavor through air influenced stress level of post-weaning piglets (Oostindjer et al., 2011).

Prenatal exposure and re-exposure of SA flavor group showed improvement 46.67% of uniformity than control group at 13 week. There is no clear evidence about relationship between uniformity changing and prenatal exposure. However, Hyun et al. (1998) reported that multiple stress factors and growth performance during growing period. Several stress factors depressed ADG, ADFI, and G:F ratio by 30.8, 15.1, and 17.5%, respectively (Hyun et al.,1998). This result showed that stress reduction through re-exposure of SA flavor affected piglet uniformity. Additional research required for making clear understanding about relationship between uniformity and prenatal flavor learning process.

Cortisol is a steroid hormone produced by the adrenal gland and released in response to stress (Jang, 2016). This study showed that prenatal exposure of SA flavor through maternal diets significantly influenced concentration of cortisol in plasma. This is consistent with the study of Oostindjer et al. (2011) who reported flavor-exposed piglets tended to show a faster decrease in salivary cortisol levels after weaning. According to the research, initial stress response was similar for all progeny at weaning time, however prenatal exposed piglets recovered faster than control group.

The less pH of pork on 24 hour postmortem of carcass as prenatal exposure of SA flavor through maternal diets observed. According to the literature (Kim et al., 1998), initial pH of meat retained pH 7.0~7.3 after slaughter. However, pH decreased as time passed and reached and retained pH 5.4~5.6. Most important indicator in pH change is speed of change and final pH of meat. In this study, pH change and final pH values were normal range what described before. Also, this study observed several

significant differences from color measurements. However, it was not consistent as time passed. According to the Buckley et al. (1995), supplementation antioxidant influenced the Hunter a values and TBARS. Although several studies reported on the effect of SA (Padmashree et al., 2007; Wang et al., 2011; Sá et al., 2017), however this report did not observe antioxidant effects from pork quality. In order to demonstrate antioxidant activity of SA in pork, it is necessary to review the diet level during growing-finishing period. These pork quality results are within reasonable limits and do not indicate the effects of supplementation of SA during growing-finishing period.

5. CONCLUSION

Large numbers of environmental factors are changed regularly during growing-finishing period. These environmental factors can be potential constraints that can limit the growth performance of pigs. Numerous studies conducted to increase growth performance through stress reduction from prenatal learning process of flavor and re-exposure same flavor during post-weaning period. In this study showed that supplementation SA during late gestation and re-exposure of SA flavor or supplementation improve growth performance of growing-finishing pigs without negative impacts of pork quality.

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Table 1. The formulas and chemical composition of experimental diet (gestation and lactation)[1]

	Gestation		Lactation
Item	**Control**	**Anise**	**Control**
Ingredient, %			
Corn, yellow	72.55	72.55	63.7
Soybean meal, 45% CP	14.75	14.75	27.2
Wheat mill run	6.0	6.0	2.8
Animal fat	2.6	2.6	2.8
Monodicalcium phosphate	1.8	1.8	1.5
Limestone	1.3	1.3	1.3
Lysine sulfate, 51%	0.29	0.29	0.1
Salt	0.3	0.3	0.3
Choline chloride, 50%	0.1	0.1	0.1
L-threonine, 98%	0.1	0.1	
Anise Extract		0.1	
Vit. Mix.[2]	0.06	0.06	0.05
Min. Mix.[3]	0.15	0.15	0.15
Total	100.0	100.1	100.0
Chemical composition[4]			
ME, kcal/kg	3075	3075	3116.4
Crude protein, %	13.0	13.0	17.7
Calcium, %	0.85	0.85	0.83
Phosphorus, %	0.71	0.71	0.67
Lysine, %	0.79	0.79	1.02
Methionine+cysteine, %	0.49	0.49	0.63
Threonine, %	0.57	0.57	0.66
Tryptophan, %	0.15	0.15	0.22

[1]Treatments: Gestation diets were fed 2.4kg/day in two separate meals; lactation diets were fed *ad libitum* up to weaning at 21days. Star anise diets were supplemented with 0.1% anise extract.

[2]Provided per kg of diet:

Gestation: vitamin A, 10,800 IU; vitamin D_3, 2,400 IU; vitamin E, 72 IU; vitamin K, 3.6 mg; vitamin B_2, 7.2 mg; vitamin B_6, 4.8 mg; vitamin B_{12}, 30 μg; pantothenic acid, 24 mg; biotin, 324 μg; niacin, 48 mg; folic acid 3.12 mg; thiamine, 1.56mg.

Lactation: vitamin A, 9,000 IU; vitamin D_3, 2,000 IU; vitamin E, 60 IU; vitamin K, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 25 μg; pantothenic acid, 20 mg; biotin, 270 μg; niacin, 40 mg; folic acid 2.6 mg; thiamine, 1.3 mg.

[3]Provided per kg of diet: Fe, 165 mg; Mn, 60 mg; Zn, 99 mg; Cu, 7.5 mg; Se, 450 μg; I, 1 mg.

[4]Calculated values.

Table 2. The formulas and chemical composition of experimental diet (grower and finisher)[1]

	Treatments			
	Grower		Finisher	
Item	Control	Star anise	Control	Star anise
Ingredient, %				
Corn, yellow	44.753	44.733	47.166	47.146
Soybean meal, 45% CP	18.14	18.14	13	13
Wheat flour	4	4	2	2
Wheat	9.8	9.8	12.8	12.8
Rice bran high fat	6	6	5.5	5.5
Sugarcane molasses	3.4	3.4	3	3
Animal fat	3.7	3.7	3.3	3.3
Meat and bone meal	1.41	1.41		
Corn gluten feed	1.5	1.5	1	1
DDGS	3	3	7.5	7.5
Palm kernel meal	1.5	1.5	2.5	2.5
Salt	0.36	0.36	0.34	0.34
Tryptophan, 10%	0.034	0.034	0.034	0.034
Mono sodium glutamate	0.1	0.1		
Zinc oxide, 90%	0.233	0.233		
Choline Chloride, 50%	0.08	0.08		
DL-methionine, 98%			0.019	0.019
L-threonine, 98%	0.115	0.115	0.096	0.096
Saccharin hydrate	0.015	0.015		
Methionine hydroxy analogue,84%	0.076	0.076		
Lysine sulphate, 51%	0.536	0.536	0.527	0.527
Organic acids	0.2	0.2		
Limestone	0.76	0.76	1.02	1.02
Enzyme mix	0.041	0.041	0.041	0.041
Star anise		0.02		0.02
Yucca powder	0.007	0.007	0.007	0.007
Yeast cell	0.04	0.04		
Butter flavor			0.02	0.02
Vit. Mix.[2]	0.1	0.1	0.05	0.05
Min. Mix.[3]	0.1	0.1	0.08	0.08
Total	100	100	100	100
Chemical composition[4]				
ME, kcal/kg	3269.1	3267.9	3257	3255.8
Crude protein, %	16.99	16.98	15.00	15.00
Calcium, %	0.53	0.53	0.5	0.5
Phosphorus, %	0.47	0.47	0.43	0.43
Lysine, %	1.09	1.09	0.93	0.93
Methionine+cysteine, %	0.66	0.66	0.58	0.58
Threonine, %	0.71	0.71	0.62	0.62
Tryptophan, %	0.19	0.19	0.16	0.16

[1]Treatments: The first factor was SA supplementation (0% or 0.1%) in late-gestation period of sows and the second factor was SA supplementation (0% or 0.02%) in growing-finishing period

[2]Provided per kg of diet:

Grower: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 60 IU; vitamin K, 3.5 mg; vitamin B_2, 8 mg; vitamin B_6, 2 mg; vitamin B_{12}, 35 μg; pantothenic acid, 25 mg; biotin, 100 μg; niacin, 50 mg; folic acid 1.1 mg; thiamine, 1.5mg.

Finisher: vitamin A, 5,000 IU; vitamin D_3, 1,000 IU; vitamin E, 30 IU; vitamin K, 1.75 mg; vitamin B_2, 4 mg; vitamin B_6, 1 mg; vitamin B_{12}, 17.5 μg; pantothenic acid, 12.5 mg; biotin, 50 μg; niacin, 25 mg; folic acid 0.55 mg; thiamine, 0.75mg.

[3]Provided per kg of diet:

Grower: Fe, 100 mg; Mn, 50 mg; Zn, 50 mg; Cu, 80 mg; Se, 400 μg; I, 1 mg.

Finisher: Fe, 80 mg; Mn, 40 mg; Zn, 40 mg; Cu, 64 mg; Se, 304 μg; I, 0.8 mg.

[4]Calculated values.

Table 3. Effect of star anise supplementation and imprinting impacts on growth performance in growing-finishing pigs

Gestation	Control		Anise 0.1%			P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise	SEM[1]	Gestation	G-F Level	G×L
Body weight, kg								
Initial	24.68	24.66	24.67	24.66				
3 week	37.46	39.13	38.36	38.19	1.067	0.9	0.75	0.69
7 week	62.05	62.86	63.26	62.71	2.033	0.6	0.90	0.92
10 week	82.08	82.99	83.79	83.08	1.516	0.7	0.98	0.81
13 week	102.49	104.76	107.73	103.52	1.501	0.5	0.76	0.31
ADG, g								
0-3 week	621	689	625	612	22.4	0.6	0.76	0.26
4-7 week	878	847	889	848	28.1	0.3	0.18	0.85
8-10 week	954	949	977	970	20.0	0.6	0.89	0.97
11-13 week	1,005	1,037	1,140	974	24.0	0.4	0.13	0.03
0-7week	763	780	788	763	25.3	0.4	0.85	0.90
8-13 week	963	998	1,059	971	15.3	0.2	0.35	0.04
0-13 week	855	880	913	859	11.9	0.4	0.54	0.11
ADFI, g								
0-3 week	1,615	1,762	1,662	1,699	57.2	0.9	0.46	0.66
4-7 week	2,360	2,400	2,358	2,302	66.5	0.5	0.59	0.76
8-10 week	3,493	3,720	3,618	3,558	70.0	0.9	0.58	0.34
11-13 week	3,745	3,783	3,564	3,558	56.4	0.0	0.89	0.85
0-7week	2,041	2,126	2,060	2,044	68.4	0.7	0.93	0.77
8-13 week	3,619	3,751	3,591	3,558	52.5	0.3	0.65	0.46
0-13 week	2,769	2,876	2,766	2,702	47.1	0.3	0.83	0.39
Gain to feed ratio								
0-3 week	0.384	0.393	0.393	0.362	0.3831	0.4	0.48	0.19
4-7 week	0.374	0.355	0.377	0.376	0.0089	0.5	0.32	0.84
8-10 week	0.276	0.255	0.271	0.272	0.0053	0.5	0.42	0.34
11-13 week	0.270	0.277	0.323	0.274	0.0089	0.1	0.19	0.12
0-7week	0.374	0.368	0.383	0.379	0.0066	0.4	0.67	0.83
8-13 week	0.267	0.266	0.296	0.273	0.0045	0.0	0.14	0.17
0-13 week	0.309	0.306	0.331	0.319	0.0039	0.0	0.28	0.51

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

Table 4. Effect of star anise supplementation and imprinting impacts on coefficient of variation (CV) in growing-finishing pigs

Gestation	Control		Anise 0.1%		SEM[1]	P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise		Gestation	G-F Level	G×L
Initial	5.23	5.29	3.27	5.76	0.386	<0.01	0.76	0.70
3 week	9.75	8.41	4.80	7.21	0.784	0.04	0.72	0.21
7 week	11.76	9.99	6.22	7.07	0.888	0.01	0.77	0.42
10 week	12.07	8.46	6.40	6.68	0.929	0.04	0.34	0.26
13 week	11.72	8.56	5.60	6.25	0.967	0.05	0.34	0.44

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

Table 5. Effect of star anise supplementation and imprinting impacts on cortisol and SOD in growing-finishing pigs

Gestation	Control		Anise 0.1%			P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise	SEM[1]	Gestation	G-F Level	G×L
Cortisol, μg/dL								
Initial	6.05	6.05	4.03	4.03	0.211			
3 week	2.82	2.60	3.90	4.95	0.370	0.02	0.54	0.36
6 week	3.93	4.22	4.13	5.58	0.499	0.45	0.41	0.58
9week	6.00	4.55	5.23	4.62	0.406	0.68	0.23	0.62
12 week	8.35	11.17	7.50	5.60	0.733	0.02	0.73	0.08
SOD[3], U/mL								
Initial	3.00	3.00	3.81	3.81	0.084			
3 week	2.25	2.58	3.63	2.72	0.205	0.06	0.45	0.12
6 week	3.23	2.95	3.02	3.31	0.133	0.79	0.98	0.33
9week	2.23	3.14	3.70	4.36	0.244	<0.01	0.08	0.75
12 week	3.49	3.41	3.44	3.40	0.107	0.91	0.80	0.93

[1] Standard error of means.
[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.
[3] superoxide dismutase.

Table 6. Effect of star anise supplementation and imprinting impacts on pork pH changes after 24hrs slaughter

Gestation	Control		Anise 0.1%			P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise	SEM[1]	Gestation	G-F Level	G×L
Time after slaughter								
0 hour	6.15	6.21	6.14	6.13	0.023	0.32	0.61	0.41
3 hour	5.77	5.69	5.74	5.76	0.043	0.81	0.75	0.60
6 hour	5.52	5.64	5.63	5.58	0.029	0.66	0.52	0.16
12 hour	5.80	5.76	5.77	5.72	0.014	0.28	0.12	0.88
24 hour	5.95	5.90	5.86	5.85	0.017	0.03	0.24	0.52

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

Table 7. Effect of star anise supplementation and imprinting impacts on pork color changes after 24hrs slaughter

Gestation	Control		Anise 0.1%		SEM[1]	P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise		Gestation	G-F Level	G×L
Hunter value, L[3]								
0 hour	39.89	38.88	38.83	40.21	0.295	0.81	0.75	0.05
3 hour	38.89	38.62	39.10	39.50	0.313	0.42	0.92	0.62
6 hour	41.51	40.95	41.28	41.74	0.384	0.74	0.95	0.54
12 hour	44.02	43.55	43.37	44.22	0.294	0.99	0.75	0.29
24 hour	45.66	45.15	44.45	45.85	0.296	0.66	0.45	0.12
Hunter value, a[4]								
0 hour	4.18	3.87	4.20	3.56	0.109	0.49	0.03	0.44
3 hour	4.15	4.16	4.35	3.99	0.104	0.96	0.42	0.40
6 hour	4.75	4.53	4.84	4.41	0.157	0.98	0.34	0.74
12 hour	5.25	5.15	5.55	5.30	0.147	0.47	0.57	0.81
24 hour	6.59	6.12	5.61	6.43	0.195	0.39	0.66	0.11
Hunter value, b[5]								
0 hour	3.33	3.01	3.17	3.07	0.081	0.75	0.21	0.50
3 hour	3.54	3.45	3.56	3.54	0.069	0.72	0.68	0.82
6 hour	4.09	3.85	4.03	3.91	0.094	0.98	0.38	0.77
12 hour	4.32	4.22	4.47	4.59	0.087	0.15	0.95	0.53
24 hour	5.64	5.11	4.82	5.67	0.127	0.55	0.46	<0.01

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

[3] L-luminance or brightness (vary from black to white).

[4] a-red·green component (+a=red, -a=green).

[5] b-yellow·blue component (+b=yellow, -b=blue).

Table 8. Effect of star anise supplementation and imprinting impacts on proximate analysis and physiochemical property of LM

Gestation	Control		Anise 0.1%		SEM[1]	P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise		Gestation	G-F Level	G×L
Proximate analysis, %								
Moisture	71.74	72.53	72.32	72.69	0.290	0.55	0.35	0.74
Crude protein	26.01	24.78	24.16	24.36	0.273	0.03	0.31	0.16
Crude fat	1.35	1.54	1.67	2.14	0.108	0.03	0.10	0.48
Crude ash	1.37	1.20	1.27	1.21	0.042	0.60	0.18	0.51
Physiochemical property								
Cooking loss, %	29.28	34.52	30.45	30.64	1.234	0.59	0.29	0.32
Shear force, kg/0.5inch	69.91	70.26	67.50	59.97	3.065	0.33	0.58	0.54
WHC[3], %	70.27	71.20	68.91	71.16	0.845	0.70	0.38	0.71

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

[3] Water holding capacity.

Table 9. Effect of star anise supplementation and imprinting impacts on fatty acid composition of LM

Gestation	Control		Anise 0.1%			P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise	SEM[1]	Gestation	G-F Level	G×L
Fatty acid composition, %								
C14:0	1.12	1.15	1.12	1.10	0.037	0.62	0.84	0.52
C16:0	21.06	20.89	20.74	20.92	0.369	0.70	0.99	0.65
C16:1	2.30	2.36	2.28	2.47	0.108	0.66	0.27	0.57
C18:0	12.15	12.22	11.84	12.03	0.254	0.34	0.61	0.82
C18:1n9c	35.03	35.20	33.92	33.93	0.822	0.16	0.91	0.93
C18:1n7	3.82	3.75	3.78	3.79	0.148	1.00	0.85	0.81
C18:2n6c	17.98	17.67	19.19	18.51	0.724	0.17	0.50	0.80
C20:0	0.10[b]	0.13[a]	0.13[ab]	0.12[ab]	0.008	0.39	0.17	0.01
C18:3n6	0.12	0.13	0.15	0.16	0.013	0.06	0.66	0.81
C20:1	0.48[b]	0.88[a]	0.86[a]	0.78[a]	0.031	<0.01	<0.01	<0.01
C20:2	0.36	0.39	0.35	0.34	0.019	0.19	0.51	0.28
C20:3n6	0.61	0.57	0.54	0.64	0.036	0.98	0.40	0.08
C20:4n6	4.76	4.52	4.96	5.07	0.287	0.20	0.82	0.55
C22:6n3	0.13	0.14	0.13	0.14	0.015	0.88	0.69	0.90
ΣSFA[2]	34.42	34.39	33.83	34.17	0.522	0.44	0.77	0.73
ΣMUFA[3]	41.62	42.19	40.84	40.96	0.893	0.28	0.70	0.81
ΣPUFA[4]	23.96	23.42	25.33	24.86	0.980	0.17	0.61	0.97

[1] Standard error of means.
[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.
[3] SFA, Saturated fatty acids.
[4] MUFA, Monounsaturated fatty acids.
[5] PUFA, Polyunsaturated fatty acids.
[a,b] Different letters within the row differ significantly (P<0.05).

Table 10. Effect of star anise supplementation and imprinting impacts on TBARS after slaughter

Gestation	Control		Anise 0.1%			P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise	SEM[1]	Gestation	G-F Level	G×L
Storage days								
0 day	0.30	0.30	0.30	0.31	0.006	0.45	0.66	0.64
3 day	0.23	0.28	0.28	0.28	0.008	0.04	0.11	0.13
7 day	0.34	0.35	0.36	0.34	0.007	0.80	1.00	0.44

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

Table 11. Effect of star anise supplementation and imprinting impacts on pork sensory evaluation of LM

Gestation	Control		Anise 0.1%			P-value[2]		
Growing-Finishing	0% Anise	0.02% Anise	0% Anise	0.02% Anise	SEM[1]	Gestation	G-F Level	G×L
Color	5.30	5.27	5.30	5.34	0.206	0.87	1.00	0.87
Flavor	5.23	5.23	5.40	5.40	0.192	0.39	1.00	1.00
Taste	5.27	5.23	5.23	5.43	0.246	0.73	0.74	0.64
Tenderness	5.23	4.87	4.63	5.43	0.306	0.96	0.48	0.07
Juiciness	4.57	4.03	4.30	4.67	0.261	0.49	0.75	0.09
Overall acceptability	5.10	4.83	4.80	5.37	0.273	0.67	0.59	0.14

[1] Standard error of means.

[2] Gestation: SA 0.1% supplementation or not during gestation, G-F Level: SA 0.02% supplementation or not during growing to finishing period, G x L: interaction between SA supplementation during gestation and growing to finishing period.

Chapter VI.Overall Conclusion

As an economic animal, productivity of pigs is very close related to the profit of pork producers. Numerous studies have been conducted to improve productivity of pigs. As one of the ways to increase pig productivity, many nutritionists considered phytogenic feed additives. Effects of phytogenic feed additives are well defined from several previous studies. However, there was very few evidence in the effects of imprinting on sow performance and growth performance of their progeny. The objectives of these experiments were 1) to investigate the effects of dietary SA supplementation as a phytogenic feed additive during gestation and lactation on the performance of multiparous sows and their progeny until 21 days post-weaning, 2) to evaluate the effects of dietary SA supplementation during late gestation and post-weaning on the performance of multiparous sows and growth performance of their progeny, and 3) to investigate the effects of SA supplementation growing to finishing pigs after prenatal exposure of SA during late gestation on growth performance and meat quality in pigs.

Supplementation of SA in the sow diet during late gestation and lactation enhanced oxidative status of sows. And SA supplementation influenced composition of sow milk at 21d lactation. This result showed that SA supplementation in the sow diet affected performance of their progeny and reduced stress related indicators at weaning and certain conditions. This report also indicated that supplementation SA during late gestation and re-exposure of SA flavor or supplementation enhanced uniformity of growing-finishing pigs and gain to feed ratio without negative impacts of pork quality.

These results implied that SA supplementation in sow diets during prenatal and postnatal period and re-exposure of SA flavor could help to increase sow productivity and growth performance of their progeny.

Chapter VII. Summary in Korean

본 실험은 모돈 사료 내 스타아니스(Star Anise; *Illicium verum*) 의 첨가가 모돈 및 자돈의 성적에 미치는 영향을 규명하기 위해 시행되었다. 총 3개의 주제로 실험을 수행하였으며 구성은 다음과 같다. 1) 분만 전후 모돈 사료 내 스타아니스의 첨가가 모돈의 번식 성적 및 이유자돈의 성장성적에 미치는 영향, 2) 임신 후기 임신돈 사료 및 이유 후기 자돈 사료 내 스타아니스 첨가가 모돈의 번식 성적 및 이유 후기 자돈의 성장성적에 각인효과로서 미치는 영향 3) 임신 후기 임신돈 사료 및 육성-비육돈 사료 내 스타아니스 첨가가 육성-비육돈의 성장 성적 및 육질에 미치는 영향.

Experiment I. Effects of Dietary Star Anise (*Illicium verum*) Supplementation during Late Gestation and Lactation on the Performance of Multiparous Sows and Their Progeny until 21 Days Post-weaning

본 연구는 분만 전후 모돈 사료 내 스타아니스의 첨가가 모돈의 번식 성적 및 이유자돈의 성장성적에 미치는 영향을 규명하기 위해서 수행되었다. 2원 교잡 [Yorkshire × Landrace] 임신 모돈 40두를 공시하여, 임신 90일령에 체중, 등지방, 산차를 고려하여 완전임의배치법(CRD: completely randomized design)으로 스타아니스의 첨가 유무 (0.1% 또는 무첨가)에 따라 처리구를 설정하여 각각 배치하였다. 각인효과에 의한 이유자돈의 성장성적을 보기 위해 포유기 이후에 160마리의 이유자돈을 공시하였으며, 0.05%의 스타아니스 첨가 유무에 따라 처리구를 설정하였다. 임신 후기 스타아니스 급여 처리구에서 혈중 total antioxidant capacity(TAS) 농도가 더 높게 나타났으며(P=0.03), 포유기에는 스타아니스 급여 처리구의 일당사료섭취량이 증가하는 경향을 보였다(P=0.08). 포유기에 스타아니스를 급여한 처리구의 이유자돈수가 증가하였으며,

포유 21일령의 복당 체중이 유의적으로 증가하였다 (P=0.06, P=0.04, respectively). 돈유 분석에서는 임신기 스타아니스 급여 처리구의 돈유 내 단백질 함량이 낮은 경향을 보였으나(P=0.07), 유당 함량은 유의적으로 높아지는 결과를 보였다 (P<0.01). 또한 임신기x포유기 스타아니스 급여의 상호작용효과는 돈유 내 고형분(total solid) 성분에서 경향을 보였으며, 유리지방산(free fatty acid) 성분에서 유의성이 있는 것으로 나타났다 (P=0.06, P=0.04, respectively). 혈중 스트레스 호르몬 분석에서는 임신기 모돈의 스타아니스 섭취가 포유자돈의 이유 시 혈중 코르티솔과 에피네프린 농도를 유의적으로 감소시켰으며 (P=0.01, P=0.04, respectively), 혈중 코르티솔 농도에서는 임신기x포유기 스타아니스 급여의 상호작용효과가 나타났다 (P=0.01). 이유 후 자돈의 성장성적에서는 임신기 스타아니스 급여 처리구의 1-2주차 사료효율(G:F ratio)이 증가하는 경향을 나타냈으며(P=0.08), 2-3주와 전체 사료효율에서 유의적으로 증가하였다 (P=0.03, P=0.05, respectively). 이유자돈의 혈액 분석에서는 임신기 및 포유기 스타아니스 처리구에서 이유 및 3주차 혈중 superoxide dismutase(SOD) 농도가 유의적으로 증가하였으며 (P<0.01, respectively), 임신기x포유기 고도의 상호작용효과가 있는 것으로 나타났다 (P<0.01). 결과적으로, 이 실험은 임신기와 포유기 모돈사료 내 스타아니스의 첨가가 모돈의 혈중 항산화 특성을 높여주고, 돈유의 유당과 유리지방산의 함량을 높여주어 복당 체중 증가와 이유스트레스 감소에 긍정적으로 영향을 미쳤으며, 이유 후 항산화 작용으로 자돈의 사료효율을 높여주는 효과를 나타낸 것으로 사료된다.

Experiment II. Effects of Star Anise (*Illicium verum*) Supplementation during Late Gestation and Post-weaning on Performance of Sow and Their Progeny

본 연구는 임신후기 임신돈 사료 및 이유자돈 사료 내 스타아니스의 첨가가 모돈의 번식 성적 및 이유자돈의 성장성적에 미치는

영향을 규명하기 위해서 수행되었다. 2원 교잡 [Yorkshire × Landrace] 임신 모돈 50두를 공시하여, 임신 90일령에 체중, 등지방, 산차를 고려하여 완전임의배치법(CRD: completely randomized design)으로 스타아니스의 첨가 유무(0.1% 또는 무첨가)에 따라 각각 배치하였다. 이유 자돈의 각인효과에 따른 성장성적을 알아보기 위해 포유기 이후에 총 120마리의 이유자돈을 공시하였다. 이유자돈들은 스타아니스 0.02% 처리구와 0.04% 처리구로 각각 배치하였다. 임신 후기 모돈의 생리반응에서는 스타아니스 급여에 따른 유의적 차이는 발견되지 않았다. 포유기에서도 총 자돈수, 복당 체중 변화, 자돈의 체중변화는 유의차가 발견되지 않았다. 임신기에 스타아니스를 급여하지 않은 대조구는 포유 21일차의 돈유의 지방과 총 고형분(total solid)이 대조구에 비해 높게 나타났다(P=0.01, respectively). 0.04% 스타아니스를 급여한 이유자돈들의 일당평균증체량이 2에서 4주 사이에 다른 처리구에 비해 높은 경향을 보였다(Weaning, P=0.09). 추가적으로 임신기에 스타아니스를 급여한 처리구의 자돈들의 일당평균섭취량이 실험 개시에서 1주 사이에 다른 처리구에 비해 낮은 경향을 보였다(Gestation, P=0.09). 0.02% 스타아니스를 급여한 이유자돈들의 사료효율이 0에서 1주차에 다른 처리구에 비해 높은 통계적 유의차를 보였다(Weaning, P=0.02). 임신기에 스타아니스를 급여한 처리구의 이유자돈의 균일도가 이유시점과 이유 후 4주차에 매우 향상된 결과를 나타냈다(P=0.02, P<0.01, respectively). 실험 4주차의 이유자돈에서 임신기에 스타아니스를 통해 각인효과가 있는 처리구의 자돈들이 낮은 혈중 코르티졸 농도를 나타났다(P<0.01). 위의 실험결과를 통해 임신후기 스타아니스의 급여는 이유 후 자돈들이 동일한 향을 다시 맡게 되었을 때 스트레스 저감에 효과적으로 작용하여 체중 균일도 향상 등의 긍정적인 효과를 나타냈으며, 0.02% 이하로 사료에 급여하는 것이 각인효과 극대화에 긍정적인 영향을 미치는 것으로 사료된다.

Experiment III. Effects of Star Anise (*Illicium verum*) Supplementation during Growing to Finishing Periods after Prenatal Exposure of Star Anise on Growth Performance and Meat Quality in Pigs

본 연구는 임신후기 임신돈 사료 및 육성·비육돈 사료내 스타아니스의 첨가가 육성·비육돈의 성장성적과 육질에 미치는 영향을 규명하기 위해서 수행되었다. 이유자돈기 사양실험 종료 후(이유 후 4 주), 2 주간의 일반사양을 한 돼지들을 대상으로 육성-비육기 사양실험을 실시하였다. 평균체중 24.83±2.95 kg 인 삼원 교잡종 ([Yorkshire × Landrace] × Duroc) 육성돈 120 두를 공시하였으며, 4 처리 5 반복 돈방당 6 두씩 성별과 체중에 따라 난괴법(Randomized complete block design)으로 배치하였다. 육성-비육돈의 각인효과를 살펴보기 위해 실험설계는 2×2 factorial 디자인을 적용하였으며, 첫 번째 요인은 임신말기 임신돈 사료 내 스타아니스의 첨가유무(0% 또는 0.1%)이며, 두 번째 요인은 육성-비육기 스타아니스의 첨가유무(0% 또는 0.02%)이다. 실험 전 구간의 체중변화와 일당 섭취량에서는 유의차가 발견되지 않았으나, 11 주-13 주 와 8 주-13 주에서 임신기 x 포유기 스타아니스 급여에 따른 상호작용효과가 실험돈의 일당 증체량 증가에 영향을 미치는 것으로 나타났다(P=0.03, P=0.04, respectively). 또한, 임신기 스타아니스를 급여한 처리구의 사료효율(G:F ratio)이 8-13 주 그리고 전체 실험 기간동안 유의적으로 증가하였다(P=0.03, P=0.02, respectively). 체중의 균일도 분석에서는 임신기 스타아니스 급여 처리구와 육성-비육기 스타아니스 급여 처리구 모두 대조구보다 균일도가 46.67% 개선되는 효과를 나타냈다(Gestation, P=0.05). 혈액 분석에서는 3 주차 임신기 스타아니스 급여 처리구가 대조구보다 높은 혈중 코르티솔 농도를 나타냈으나, 실험 12 주차에서는 대조구가 다른 처리구에 비해 높은 혈중 코르티솔 농도를 보였다(P=0.02, P=0.02, respectively). 임신기에 스타아니스를 급여한 처리구는 실험 9 주차의 혈중 superoxide dismutase (SOD)가 다른 처리구에 비해 높은 농도로 검출되었다(Gestation, P<0.01). 도체 후 24 시간 내 돈육 산도 측정 결과 임신기 스타아니스 급여 처리구의 pH 가

대조구보다 유의적으로 낮은 것으로 나타났으며(Gestation, P=0.03), 돈육의 일반성분 분석에서는 임신기 스타아니스를 급여한 처리구의 조단백질은 감소한 반면 조지방은 증가하는 것으로 나타났다(P=0.03, P=0.03, respectively). 따라서, 임신말기 스타아니스 급여 후 육성-비육기에 추가적으로 스타아니스를 급여하는 경우 돼지고기의 품질에 영향을 주지 않으면서 육성·비육돈의 성장성적에 긍정적인 영향을 주는 것으로 나타났다.